Jasmonates and abscisic acid influence fruit ripening and plant water use: practical, physiological and morphological aspects

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The aim of the present thesis was to better understand the physiological role of the phytohormones jasmonates (JAs) and abscisic acid (ABA) during fruit ripening in prospect of a possible field application of JAs and ABA to improve fruit yield and quality. In particular, the effects of exogenous application of these substances at different fruit developmental stages and under different experimental conditions were evaluated. Some aspects of the water relations upon ABA treatment were also analysed. Three fruit species, peach (*Prunus persica* L. Batsch), golden (*Actinidia chinensis*) and green kiwifruit (*Actinidia deliciosa*), and several of their cvs, were used for the trials. Different experimental models were adopted: fruits *in planta*, detached fruit, detached branches with fruit, girdled branches and micropropagated plants. The work was structured into four sets of experiments as follows:

(i) Pre-harvest methyl jasmonate (MJ) application was performed at S3/S4 transition under field conditions in Redhaven peach; ethylene production, ripening index, fruit quality and shelf-life were assessed showing that MJ-treated fruit were firmer and thus less ripe than controls as confirmed by the Index of Absorbance Difference (I\_AD), but exhibited a shorter shelf-life due to an increase in ethylene production. Moreover, the time course of the expression of ethylene-, auxin- and other ripening-related genes was determined. Ripening-related ACO1 and ACS1 transcript accumulation was inhibited though transiently by MJ, and gene expression of the ethylene receptor ETR2 and of the ethylene-related transcription factor ERF2 was also altered. The time course of the expression of several auxin-related genes was strongly affected by MJ suggesting an increase in auxin biosynthesis, altered auxin conjugation and release as well as perception and transport; the need for a correct ethylene/auxin balance during ripening was confirmed.

(ii) Pre- and post-harvest ABA applications were carried out under field conditions in Flaminia and O’Henry peach and Stark Red Gold nectarine fruit; ethylene production, ripening index, fruit quality and shelf-life were assessed. Results show that pre-harvest ABA applications increase fruit size and skin color intensity. Also post-harvest ABA treatments alter ripening-related parameters; in particular, while ethylene production is impaired in ABA-treated fruit soluble solids concentration (SSC) is enhanced. Following
field ABA applications stem water potential was modified since ABA-treated peach trees retain more water.

(iii) Pre- and post-harvest ABA and PDJ treatments were carried out in both kiwifruit species under field conditions at different fruit developmental stages and in post-harvest. Ripening index, fruit quality, plant transpiration, photosynthesis and stomatal conductance were assessed. Pre-harvest treatments enhance SSC in the two cvs and flesh color development in golden kiwifruit. Post-harvest applications of either ABA or ABA plus PDJ lead to increased SSC. In addition, ABA reduces gas exchanges in *A. deliciosa*.

(iv) Spray, drench and dipping ABA treatments were performed in micropropagated peach plants and in peach and nectarine detached branches; plant water use and transpiration, biomass production and fruit dehydration were determined. In both plants and branches ABA significantly reduces water use and fruit dehydration. No negative effects on biomass production were detected.

The present information, mainly arising from plant growth regulator application in a field environment, where plants have to cope with multiple biotic and abiotic stresses, may implement the perspectives for the use of these substances in the control of fruit ripening.
1. GENERAL INTRODUCTION

Developing fruits are terminal sinks that require carbohydrate, other metabolites, mineral nutrients and adequate water to sustain growth. In addition, development of these organs is known to be affected by plant hormones which act either directly or indirectly to alter gene expression (Cowan et al., 2001). Fruit development and ripening are major steps in fruit tree production because they determine overall fruit size and quality. In most species, fruit growth can be divided in three main stages. The first one includes the ovary development inside the flower and fruit set. During the second phase, fruit grows due to cell proliferation. The final step is characterized by growth due to cell enlargement; in this phase food reserves are accumulated and most fruits attain its final shape and size. Fleshy fruits after reaching their final size and shape, ripe. This process is characterized by dramatic changes in color, texture and taste, which confers the palatable attributes that characterize them (Gillaspy et al., 1993; Pirrello at al., 2009). The physiological changes occurring in fruits during the different stages of development and ripening are mainly due to the relative levels of several hormones. Fruit set and early fruit growth are dependent on the production of auxin and gibberellins (GAs) by developing seeds, when seeds approach maturity, their importance as production centers of hormones decreases, and for further fruit development hormone supply is essential. In addition to auxin and GAs, cytokinins (CK) that are known to stimulate cell division, may also be involved in early fruit development. Finally, abscisic acid (ABA) and ethylene play key roles in developing fruits, the latter increasing especially when fruit ripening starts (Tromp and Wertheim, 2005).

1.1 Fruit growth

Flesh fruits are botanically diverse, with some like tomato and grape being true berries derived from ovary, and others such as strawberry, pineapple and apple derived from the receptacle tissues or from expansion of the sepals (Barry and Giovannoni, 2007). In general, fruit growth, as fresh weight gain or size increment, follows a sigmoidal curve (Connors, 1919; Baldini, 1986, Westwood, 1993) which can be separated in two distinct growth patterns. In the first stage, which varies upon fruit species, growth is due mainly to cell divisions and accounts for little increase in fruit size (Gillaspy et al., 1993; Westwood, 1993; Grierson, 2002; Tromp and Wertheim, 2005). Cell number is a function of the number of mitotic divisions which require the presence of indol-3-acetic acid (IAA) and CK; in the absence of CK, cells do not divide (Cowan
et al., 2001); ABA negatively regulates cell division since it inhibits cell division of maize kernels, maize root tips, pea buds and pollen cells (Reddy and Day, 2002).

In the second stage, growth is mainly due to cell expansion and accounts for a rapid increase in size due to the development of vacuoles in the newly formed cells (Grierson, 2002). During this stage, growth is enhanced by the presence of seeds since they are an important hormone production center (Cowan et al., 2001). In general, high IAA and GA levels are associated to active seed growth by cell expansion and fruit growth; these hormones are at their maximum during mid-embryo growth when CK content is rapidly declining and there is little or no ABA (Cowan et al., 2001); seedless avocado fruits are considerably smaller than the bearing-seeds ones (Cowan et al., 2001). Studies of the effect of ABA and CK on fruit size revealed that mesocarp ABA level was negatively correlated with avocado (Moore-Gordon et al., 1998) and litchi (Li et al., 2005) fruit size that is consistent with reports which state that ABA retards cell division cycle activity (Meyers et al., 1990; Mambelli and Setter, 1998).

From a biophysical point of view, fruit growth may be defined as a balance between incoming and outgoing fluxes; when this balance is positive fruit increase in weight, while in contrast when it is negative they shrink (Fishman and Génard, 1998). Carbon and water enters the fruit via phloem and xylem streams, driven by hydrostatic pressure gradients along the vascular path (Minchin and Thorpe, 1996; Patrick, 1997). In early stages of development, phloem assimilate unloading to terminal accumulating sinks occurs via symplasm (Patrick, 1997) and switches to apoplasm towards the end of growth when a grand phase of photoassimilate import begin (Lalonde et al., 2003). Symplasmic solute flow occurs through plasmodesmata, by diffusion or bulk flow, following osmotic potential gradients (Lalonde et al., 2003) and may be modulated by plant hormones (Morris, 1996) while apoplastic transport becomes necessary when no or too low water potential gradients exist, to actively transport carbohydrates into the fruit (Lalonde et al., 2003). In peaches similar relative contribution of phloem, xylem and transpiration occurs during fruit growth, with higher xylem contribution to growth throughout the season (Morandi et al., 2007). In kiwifruits, in contrast, xylem fluxes decreases and phloem flux rates are constant through the season, indicating an increase in phloem contribution to fruit growth with fruit development (Morandi et al., 2010).

Soluble sugar composition and availability strongly affect cell cycle activity and cell proliferation as demonstrated for kernel set in maize, fruit development in Trillium and fruit size in tomato (Cowan et al., 2001). Sugar utilization during organ development is a function of 2
sugar-metabolizing enzymes that are encoded by sugar-responsive genes. Also, fruit growth is influenced by isoprenoid availability since several products of the isoprenoid pathway are potentially involved in the control of cell division and fruit size (Gillaspy et al., 1993).

Stone fruit growth curve follows a double-sigmoidal pattern (Baldini, 1986, Westwood, 1993, Grierson, 2002; Tromp and Wertheim, 2005), and four distinct stages have been identified. The first one, named S1, is represented by growth gain initially due to cell division and then, towards the end, to cell elongation. The second one, called S2, is a slow growth period that coincides with pit hardening, in which lignification of the endocarp proceeds rapidly while mesocarp and seed growth is suppressed. In the S3 stage fruit growth is only due to cell elongation, and fruits reach its final size. In stage 4 (S4), fruits stop their growth and ripening-related changes begin.

In kiwifruits, diverse growth curves have been found by different researchers, though most of them coincide with a single sigmoid curve (Okuse and Ryugo, 1981; Beever and Hopkirk, 1990; Walton and De Jong, 1990; Gallego et al., 1997). In this curve, growth can be clearly divided in two phases: an initial period (less than 60 days after anthesis, DAA) where relative growth rate and diameter increase are high and slow down with time; and a second stage (60 to 180 DAA) of slow growth with low and constant relative growth rate that lasts until harvest (Beever and Hopkirk, 1990; Gallego et al., 1997; Morandi et al., 2010).

1.2 Fruit ripening

Fruit ripening can be considered as the final step of fruit differentiation. In fleshy fruits this is associated with the activation of metabolic pathways that cause flesh softening, chlorophyll degradation, pigment synthesis, sugar and organic acid content modification, antioxidant accumulation and volatile synthesis that, as a whole, confer the fruits their typical palatable attributes needed for marketing.

Fruits have been classically categorized as climacteric and non-climacteric on the basis of the presence or absence of a net increase in fruit respiration rate and a burst in ethylene production at the onset of ripening (Biale and Young, 1981). Climacteric fruits include tomato, apple, peach, melon, banana and kiwifruit, whereas strawberry, grape, citrus and sweet cherry are considered as non-climacteric. However this distinction is not absolute since closely related melon and Capsicum species can be both climacteric and non-climacteric, and some so-called non-climacteric fruits display enhanced ripening phenotypes in response to exogenous ethylene.
Nevertheless, increased ethylene synthesis at the onset of ripening is required for normal ripening of many fruits (Barry and Giovannoni, 2007).

Several studies confirm that climacteric fruits need ethylene to undergo normal maturation as demonstrated by the inhibition of the process in transgenic ACS (Oeller et al., 1991) and ACO (Hamilton et al., 1990; Ayub et al., 1996) antisense plants where ethylene production is suppressed. Moreover, the need for ethylene is demonstrated by the discovery of the never-ripe (nr) tomato mutant which can not perceive ethylene due to a mutation in the ethylene binding domain of the NR ethylene receptor (Wilkinson et al., 1995), and the ripening inhibitor (rin) tomato mutant that does not show the autocatalytic ethylene production and can not transmit the ethylene signal downstream to ripening genes due to a mutation in the RIN transcription factor (Vrebalov et al., 2002). Reduction in ACS gene expression and ethylene production also seems to be responsible for the non-ripening phenotype in peach cultivars that carry the recessive stony hard (sh) mutation (Tatsuki et al., 2006), where sh fruits fail to soften on the tree or in post-harvest though other ripening traits such as color development, soluble solids accumulation, and flavor characteristics are fairly normal (Haji et al., 2001 in Tatsuki et al., 2006).

It is extensively demonstrated that ethylene regulates the transcription of several genes associated to ripening-induced metabolic pathways including its own autocatalytic biosynthesis (Giovannoni, 2004). The role of other plant growth regulators such as auxins, polyamines, jasmonates (JAs), ABA and brassinosteroids, on climacteric fruit ripening instead, still needs further investigations. The study of transgenic plants with repressed ethylene production demonstrated that, during maturation, genetically determined ethylene-dependent and ethylene-independent processes coexist (Bauchot et al., 1998; Flores et al., 2001). In peaches, auxin-related genes are up-regulated during the transition to ripening; these include the PIN auxin efflux facilitator, auxin response factors (ARF), Aux/IAA proteins (transcriptional modulators of the hormone response), a hormone receptor encoding gene (TIR1), and genes involved in auxin biosynthesis such as indole-3-glycerol phosphate synthase (IGPS) and tryptophan synthase (Wsynt; Trainotti et al., 2007). Recently, Zhang et al. (2009a) found increasing levels of 9-cis-epoxycarotenoid dioxygenase transcripts (NCED1) that enhanced the ABA accumulation in grapes and nectarines and preceded ethylene production at the onset of ripening; in both fruits, low levels of ABA induce physiological maturation reactions that were ethylene-independent; however, when ABA was applied exogenously together with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception (Sisler and Serek, 1997), both fruits did not ripe and soften.
While the role of ethylene in climacteric fruit ripening has been broadly studied, the hormonal mechanisms that regulate this process in non-climacteric fruits still need to be clarified (Giovannoni, 2004). In strawberry, considered the model species for non-climacteric fruits, the isolation of several genes correlated with ripening and the analysis of the gene expression profile of the receptacle revealed that the metabolic pathways that modify fruit consistency, color, aroma and flavor are similar to those activated in climacteric fruits (Aharoni and O’Connell, 2002). Ripening-related genes seem to be regulated by auxins in strawberry; indeed, a recent microarray analysis revealed that exogenous auxins may regulate positively and negatively some of the genes correlated with ripening; some other genes are independent from auxins (Aharoni et al., 2002) showing that in non-climacteric fruits different hormones are involved in ripening. Recently, several studies showed that exogenous ethylene accelerates non-climacteric fruit ripening, evidencing that this hormone can play a role in this process. In fact, ethylene stimulates anthocyanin biosynthetic gene expression (El-Kereamy et al., 2003), and alcohol dehydrogenase transcript levels in grape (Tesniere et al., 2004) whereas in citrus stimulates fruit de-greening without altering other ripening related processes (Katz et al., 2004).

1.2.1 Ethylene

The gas ethylene is synthesized in many tissues in response to stress, in particular in those undergoing senescence or ripening (Davies, 2010). Its biosynthesis starts from methionine and consists of 3 steps: a) conversion of methionine to S-adenosyl-L-methionine (SAM) which is catalyzed by the enzyme SAM synthase, b) formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM via ACC synthase (ACS) activity, and c) the conversion of ACC to ethylene as catalyzed by ACC oxidase (ACO). In addition to ACC, ACS forms 5’-methylthioadenosine (MTA) which is utilized to form more methionine via the Yang cycle (Pech et al., 2010). The formation of ACC and its conversion to ethylene are the two committed steps in the hormone synthesis as the Yang cycle maintain high levels of biosynthesis even under low methionine availability (Kende et al., 1993).

In plants, two systems of ethylene production have been defined. System 1 is auto-inhibitory, characterized by a negative feedback of ACS and ACO such that exogenous ethylene inhibits its synthesis, and inhibitors of ethylene action can stimulate ethylene production; system 1 functions during normal growth and development, during stress responses and even in pre-climacteric
stages of fruit development. System 2 is auto-catalytic, characterized by a positive feedback of ACS and ACO, and is stimulated by ethylene and suppressed by inhibitors of ethylene action; it operates during senescence and fruit ripening (Lelievre et al., 1997; Barry and Giovannoni, 2007). ACS and ACO are encoded by multi-gene families in higher plants. In tomato at least 9 ACS and 3-4 ACO genes exist, and among them 4 ACS and 3 ACO genes are differentially expressed in tomato fruits (Barry and Giovannoni, 2007). LeACS6 and LeACS1A are expressed during the pre-climacteric stage, and LeACS2 in the climacteric one; during transition to climacteric LeACS6 is silenced and LeACS1a and LeACS4 are up-regulated (Barry et al., 2000); LeACO1 and LeACO3 instead are expressed during fruit maturation and contribute to auto-catalytic ethylene synthesis (Barry et al., 1996; Nakatsuka et al., 1998). LeACS2 and LeACS4 are not expressed in rin (ripening inhibitor) and nr (non-ripening) tomato mutants (Pech et al., 2010).

Peach fruit ripening also relies on changes in expression of several genes, including ACS and ACO, the key enzymes of the ethylene biosynthetic pathway. ACO1 is expressed in flower, fruitlet abscission zones, mesocarp, and in young fully expanded leaves, and its transcript accumulation strongly increases during fruitlet abscission, in ripe mesocarp and senescent leaves (Rasori et al., 2003).

1.2.2 Cell wall metabolism

Fruit flesh consistency depends on cell adhesion and cell wall rigidity (Pitt and Chen, 1983). In dicot, cell wall consists of rigid, inextensible cellulose microfibrils held together by interpenetrating coextensive networks of matrix glycans, pectins and structural glycoproteins. The microfibrils are held together with glycans and pectins by covalent and electrostatic bonds (Brummell and Harpster, 2001).

During ripening, cell wall architecture and composition are progressively modified, becoming increasingly hydrated as the cohesion of the pectin gel changes. Cell wall is degraded mainly by the action of wall hydrolases such as endo-β-glucanase (EGase), exo- and endo-polygalacturonase (exo- and endo-PG), β-galactosidase (β-gal), pectin methylesterase (PME), and expansins (EXP). The moment when cell-to-cell adhesion decreases, via the breakdown and dissolution of the pectin rich middle lamella, will determine the characteristic flesh texture of diverse species; this occurs early in ripening of soft fruits as tomato and late in crisp fruit as apple (Brummell and Harpster, 2001).
1.2.3 Sugars and organic acids

Carbohydrate and organic acid content have a notable importance in defining fruit organoleptic properties as the acid/sugar ratio defines fruit flavor, and carbon metabolism is strictly correlated with the pathways of secondary metabolite synthesis, such as pigments, vitamins and volatiles. Acids, alcohols, aldehydes, esters and lactones, responsible for fruit aroma, and terpenes, that are also precursors of lycopene and carotenoids, are derived from fatty acid degradation.

Intermediates formed during glycolysis can be used in other metabolic pathways. For example, from phosphoenolpyruvate (PEP), through the shikimic acid cycle, aromatic aminoacids as tyrosine, tryptophan and phenylalanine are synthesized. The last one is used as a precursor in the biosynthesis of phenolic components such as lignine, flavonoids and anthocyanins. Furthermore, from pyruvate the aminoacids leucine, valine and isoleucine are formed, and branched-chain volatiles are derived from their catabolism (Carrari and Fernie, 2006).

In higher plants, sucrose is the main form of transport carbohydrate. Sucrose use in sink tissues depends on sugar transport from apoplast, through cytosol, to vacuoles (Nguyen-Quoc and Foyer, 2001). In this process, sucrose is degraded in glucose and fructose by sucrose synthase (SuSy) or by invertases (Koch, 2004), and resynthesized by SuSy or sucrose phosphate synthase (SPS). SuSy and SPS are cytoplasmic enzymes, while the invertases are localized in the cell wall, cytoplasm and vacuoles (Nguyen-Quoc and Foyer, 2001). In general, hexoses favor cell division and sucrose favors differentiation and maturation (Koch, 2004). During fruit development, the fruit passes from fast carbohydrate utilization to carbohydrate accumulation indicating a change in sugar metabolism, thus shifting the enzymatic activity of sugar metabolism and phloem unloading (Nguyen-Quoc and Foyer, 2001). In tomato, during the fast growth phase, sucrose unloading occurs via symplasm and is regulated by SuSy and SPS. In the following steps, sucrose unloading occurs via apoplasm; in this case wall invertase catabolizes sucrose in fructose and glucose, and they are imported by a hexose transporter inside the cell, where sucrose is re-synthesized by SuSy and SPS (Nguyen-Quoc and Foyer, 2001). Invertases mediate the initiation and expansion of many new sink structure, while transition to storage and maturation phases is facilitated by changes in hexose/sucrose ratio, thus by a shift from invertase to sucrose-synthase paths of sucrose cleavage (Koch, 2004).
General Introduction

In peach fruits, glucose and fructose are predominant in the early stages of development; then their concentration decreases, while sucrose concentration exponentially increases until it becomes the principal carbohydrate in mature fruits and sorbitol remains low during the whole growth period (Moriguchi et al., 1990; Vizzotto et al., 1996; Lo Bianco et al., 1999). Lo Bianco et al. (1999) detected SuSy activity only during stage 1, invertase activity during S1 and S3 and sorbitol metabolizing enzymes during S3. Several studies show that kiwifruits accumulate starch from 40 to 60 days after full bloom (DAFB) while glucose and fructose content increases rapidly from 140 DAFB onwards and sucrose concentration rises only with the onset of net starch degradation just prior to harvest (Okuse and Ryugo, 1981; Walton and DeJong, 1990; Klages et al., 1998; Miller et al., 1998); also, an early peak in glucose occurs prior to the start of starch accumulation (Okuse and Ryugo, 1981; Walton and DeJong, 1990; Klages et al. 1998). Yellow kiwifruits exhibit a similar carbohydrate seasonal accumulation pattern (Boldingh et al., 2000) but they have higher total sugar content than green ones at eating time (Nishiyama et al., 2008).

As far as organic acids are concerned, along with sugars, they determine fruit taste by producing tartness (Nishiyama et al., 2008). The main organic acids accumulated in fruits are malic and citric acid (Tucker, 1993). Their content depends on the accumulation in the early stages of fruit development (Laval-Martín et al., 1977), vacuolar compartmentalization (Muller et al., 1996) and mobilization during ripening (Ruffner et al., 1982). The key enzyme in acid synthesis is phosphoenolpiruvate carboxylase (PEPC; Moing et al., 2000) whereas malic acid degradation during ripening is carried out by malic (ME) and malate dehydrogenase enzymes (MDH), and citric acid degradation by isocitrate dehydrogenase (ICDH; Ruffner, 1982; Popova and Pinheiro de Carvalho, 1998). In ripe peaches, malic, citric and quinic acids are present (Moing et al., 1998). Malic acid accumulates mainly during the fast growth S1 stage, whereas citric acid accumulates during S3 stage (Zanchin et al., 1994). Peaches with low acidity do not accumulate acids during development (Moing et al., 1998). At harvest, both green and yellow kiwifruits mainly contains citric (40-60 %) and quinic acids (40-60%), and, to a lesser extent, malic acid (10-20%) with differences among cultivars (Marsh et al., 2007; Nishiyama et al., 2008).
1.2.4 Volatile synthesis

Ripening is accompanied by the production of several volatile compounds, such as terpenes, esters, alcohols, aldehydes and lactones that define fruit aroma (Lalel et al., 2003; Kondo et al., 2005; Wang et al., 2011). Terpenes derive from pyruvate and glyceraldehyde-3-phosphate, and esters, alcohols and linear-chain aldehydes from acetyl coenzyme A which is formed during glucose and fatty acid catabolism. Branch-chain esters come from the catabolism of branched amino acids (Dudareva et al., 2004). Fruits also produce acetaldehyde and ethanol by alcoholic fermentation (Pesis, 2005) during ripening. The quantity and quality of volatiles produced during ripening is characteristic of the fruit species. In peach and nectarine, a hundred of volatile compounds that contribute to fruit aroma have been identified. Esters, aldehydes and C₆ alcohols are present in unripe fruits, while, during ripening, their concentration decreases concomitant to an increase in linalool, benzaldehyde, and γ- and δ-lactones that confer the typical peach aroma (Visai and Vanoli, 1997). On-tree peach ripening goes along with a rise in acetaldehyde, ethanol and acetate ester production (Lavilla et al., 2001). In kiwifruits there is a rise in ester and alcohol content while aldehydes decrease during fruit softening (Wang et al., 2011).

1.2.5 Pigment synthesis

In most fruits, as ripening advances, a progressive exocarp and mesocarp de-greening occurs due to the conversion of chloroplast in cromoplast. Thylakoids, starch deposits and chlorophyll degrade progressively and are substituted by carotenoids and anthocyanins (Matile et al., 1999) which are synthesized from isopentenyl diphosphate (IPP; Bramley, 2002) and through the phenylpropanoid pathway (Winkel-Shirley, 2001), respectively.

This aspect of fruit maturation is important not only from a commercial point of view, because fruit color is widely appreciated by consumers, but also from a health and nutritional point of view as carotenoids and flavonoids are molecules with elevated antioxidant power (Pietta, 2000; Sajilata et al., 2008). Quantitative and qualitative profiles of phenolic compounds vary greatly between species. In tomato peel the main flavonoid accumulated is naringenin chalcon and to a lesser extent quercetin-rutinoside (Verhoeyen et al., 2002); pome fruits are characterized by a high production of catechin, procyanidine and cinnamic acids (Nicolas et al., 1994), citrus is rich in flavanone glycoside such as eriocitrin and hesperidin (Nogata et al., 2006).
while grape accumulates quercetin and myricetin (Mattivi et al., 2006). Peach and nectarines are rich in cinnamic acid, catechin, epicatechin and procyanidine (Andreotti et al., 2008). As far as carotenoids are concerned, the most widespread in fruit is β-carotene, a precursor of vitamin A. Tomatoes mainly accumulate lycopene (Fraser et al., 1994), peaches and nectarines β-carotene and β-crixtoxantine (Gil et al., 2002), and kiwifruits β-carotene and lutein (McGhie and Ainge, 2002; Montefiori et al., 2009).

1.2.6 Agronomical determination of ripening

The parameters usually measured in fruits to specifically indicate horticultural maturity are flesh firmness, skin color, flesh color and concentration of sugars, soluble solids, total acids, chlorophyll and carotene. Fruit growers pick their crops as early as possible because prices for first picked fruits are higher, and, in the case of late season fruits, such as kiwifruits, to avoid frost and bad weather conditions (Beever and Hopkirk, 1990). However, this practice can incur in harvesting immature fruits with poor color, flavor and shelf life that will never reach an excellent eating quality (Tromp, 2005). Late picking improves fruit quality but increases the risk of mechanical damage, physiological disorder appearance and disease development, so that long-term storage is not feasible (Kader, 1999; Tromp, 2005). For peaches, ground color change and flesh firmness are widely used to establish harvest date. In green kiwifruits, a minimum soluble solid content is required in order to maximize fruit storage life, with New Zealand setting its minimum at 6.2% and California at 6.5% for cv. Hayward (Westwood, 1993; Kader, 1999); when harvested with SSC lower than 6 Hayward fruits develop flesh breakdown during storage (Crisosto and Crisosto, 2001). In yellow kiwifruits flesh color, expressed as Hue angle (H°), is the most important parameter used, with cv. Hort16A at H° lower than 103 (Woodward, 2007).

1.3 Jasmonates

JAs are plant growth regulators that belong to the family of oxygenated fatty acid derivatives, collectively known as oxylipins (Howe, 2010). JAs (Fig. 1.1); they derive from the oxidation of linolenic acid (LA), and have a key role in response to wounding and biotic and abiotic stresses; they are also involved in developmental processes as seed germination, flower development,
pollen formation, anther dehiscence, root growth, fruit ripening, and leaf abscission and senescence (Wasternack and House, 2002).

![Diagram of jasmonate structure]

Figure 1.1 Structure of the principal jasmonates.

The principal JAs are jasmonic acid and its methylester, methyl jasmonate (MJ). MJ is a volatile compound present in the essential oils of several species, such as *Jasminum grandiflorum* L.; besides acting in developing and defense processes, MJ is an important inter- and intra-plant signal (Seo *et al.*, 2001). The diverse JAs are normally present in all plant tissues in concentrations and ratios that vary upon species, tissue, development stage, and in response to external stimuli (Stinzi *et al.*, 2001).

### 1.3.1 Jasmonate biosynthesis and perception

Jasmonic acid biosynthesis (Fig. 1.2) starts with the oxidation of linoleic acid (LA) catalyzed by a lipooxygenase enzyme (LOX) which is mainly localized in the cytosol and forms 9- and 13-hydroperoxylinoleic acid (HPOT); then allene oxide synthase (AOS) transforms 13-HPOT in an instable epoxide that is cyclized by allene oxide cyclase (AOC) to form a cyclopentenone, 12-oxo-phytodieneic acid (OPDA). Finally, OPDA is reduced by OPDA reductase (OPR) to form
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(+)-jasmonic acid and (-)-jasmonic acid after 3 β-oxidations (Wasternack and House, 2002; Howe, 2010). Jasmonic acid biosynthesis occurs in two diverse compartments, with the conversion of LA to OPDA occurring in the chloroplast and the β-oxidations in the peroxisomes (Devoto and Turner, 2005); jasmonic acid metabolism which leads to the synthesis of biologically active compounds such MJ, tuberonic acid, cucurbic acid, cis-jasmone and isoleucine conjugates occurs in the cytoplasm (Wasternack and House, 2002). Four AOC and LOX genes and just one AOS have been identified in Arabidopsis (Howe, 2010) while 3 highly correlated OPR genes have been characterized in Arabidopsis and tomato (Howe, 2010). Newly formed jasmonic acid is subjected to a variety of enzymatic transformation such as methylation of C₁ that produces MJ via the carboxyl methyltransferase (JMT) enzyme (Cheong and Do Choi, 2003), hydroxylation of C₁₁ or C₁₂ that gives the tuberonic acid derivative, conjugation of carboxyl terminus to produce amino acids, reduction of C₆ yielding cucurbic acid derivative isopentenil diphosphate, and degradation of C₁ to form (Z)-jasmone (Fig. 1.3; Howe, 2010).

Figure 1.2 Octadenoïd pathway for JA biosynthesis. Modified from Howe, 2010.
Under normal developmental conditions and in defense response the enzymes that control the biosynthetic pathway of jasmonic acid are regulated by a positive feedback. Exogenous JA treatments or stress-enhanced endogenous levels stimulate the expression of LOX (1996; Heitz et al., 1997; Vörös et al., 1998; Ziosi et al., 2008a), AOS (Laudert and Weiler, 1998; Sivasankar et al., 2000; Agrawal et al., 2002; Ziosi et al., 2008a), AOC (Stenzel et al., 2003; Maucher et al., 2004), OPR (Müssig et al., 2000), and JMT (Cheong and Do Choi, 2003). AOS seems to be the key regulatory enzyme in JA biosynthesis as suggested by enhanced production of jasmonic acid in transgenic potato plants that over-express the enzyme (Harms et al., 1995), and inhibited jasmonic acid biosynthesis and male sterility in Arabidopsis after T-DNA mediated AOS disruption (Park et al., 2002). AOS transcript accumulates in all plant tissues, primarily in those with elevated endogenous JA as leaves and flowers (Laudert and Weiler, 1998; Sivasankar et al., 2000). In tomato fruits, AOS transcript levels decrease during development (Howe et al., 2000), and are positively correlated with elevated JA levels in the first stages of development (Fan et al., 1998b). It is demonstrated that AOS transcript amount is enhanced by wounding, pathogens, exogenous JAs and ethylene and is correlated with protein accumulation, increased enzymatic activity and higher JA content (Laudert and Weiler, 1998; Sivasankar et al., 2000; Agrawal et al., 2002).
Exogenous JA or environmental conditions that stimulate an increase in its endogenous content produces changes in gene expression, like inhibition of nuclear and plastidial proteins involved in photosynthesis (Reinbothe et al., 1997), and induction of genes collectively known as JA-responsive genes (JRGs) that encode JA-induced proteins (JIPs) involved in JA biosynthesis and defense mechanisms.

1.3.2 Physiological roles of jasmonates

1.3.2.1 Stress responses

The most studied aspect of JA physiology is its influence in mechanical damage (Peña-Cortés et al., 1995) and insect and pathogen responses (Thomma et al., 1998); however, it has been demonstrated that they also take part in abiotic stress responses such as hydric shortage (Lehmann et al., 1995).

Plants use constitutive and inducible defenses in response to pathogens and herbivorous. Constitutive defenses include chemical-physical barriers like cell wall, cuticle, tricoms and needles. Inducible defenses consist of noxious substance accumulation, such as phytoalexins and secondary metabolites, and volatile emission that inhibits pathogen development. Mechanical and insect damages come along with the production of putative signal compounds like oligogalacturonides (OGA) from damaged walls (Orozco-Cardenas et al., 2001), reactive oxygen species (Ryan, 2000), JAs, sistemine (SYS), ABA (Peña-Cortes et al., 1995) and ethylene (O’Donnell et al., 1996).

Defense responses can be localized or systemic. It has been suggested that SYS after being produced in the wound site is loaded into the phloem and taken to undamaged leaves, thus acting as a systemic response (Nárvaez-Vásquez et al., 1995). However, in tomato, it seems that SYS is produced at or near the wound site to amplify JA biosynthesis to a level necessary for the systemic response (Howe, 2010). This supports other studies which indicate that JAs can act as a systemic signal (Li et al., 2002) as JA synthesis occurs in phloematic cells (Stenzel et al., 2003), where the biosynthetic enzymes LOX, AOS e AOC are expressed as it occurs in tomato (Hause et al., 2003). Even MJ, being a gas, can mediate systemic responses, as demonstrated by the induction of defense mechanisms on neighboring plants (Howe, 2010).
In defense responses ethylene and salicylic acid (SA) are also involved. Ethylene and JAs are both necessary for responses to fungi since insensitive mutants to ethylene and JA, ein2 and coi1, are more susceptible to fungus attacks (Lorenzo et al., 2003) while the cev1 mutant that express both transduction signals is more resistant (Ellis and Turner, 2001). Their interactions concerning herbivory depend on the species. In tomato, ethylene and JAs reciprocally induce their biosynthesis and mediate common defense responses by synergistically stimulating the expression of numerous genes (O’Donnell et al., 1996). However, in Arabidopsis, ethylene induces JA synthesis, but not the opposite, and ethylene-insensitive mutants show an increase in the expression levels of JRGs (Rojo et al., 1999). Regarding JA and SA interactions, they are mostly antagonistic as shown by the inhibition of wound-induced JA biosynthesis and AOS transcript accumulation by SA (Peña-Cortés et al., 1995); moreover, SA insensitive or SA deficient mutants have higher JRGs transcript levels than wild-type plants (Gupta et al., 2000). Jasmonic acid also acts negatively on SA, by inhibiting SA-dependent gene expression (Howe et al., 2010). In some cases, JA, SA and ethylene interaction can be synergistic, as shown by the cooperative regulation of numerous defense genes in a microarray analysis of Arabidopsis response to Alternaria (Schenck et al., 2000).

1.3.2.2 Plant development

JAs were initially described as growth inhibitors because exogenous applications inhibited seed germination, plant development and root growth (Creelman and Mullet, 1997). Arabidopsis mutants with reduced sensitivity to JAs exhibit the same features as wild-type JA-treated plants, including short roots, stunted growth and anthocyanin accumulation (Howe, 2010). However, JAs also stimulate cell division in potato and are present at high concentration in actively dividing tissues such as young fruits (Fan et al., 1997a).

During leaf senescence genes involved in JA biosynthesis are shown to be differentially up-regulated, and JA levels in senescent leaves are 4-fold higher than in non-senescent leaves (Gan, 2010). In many plants exogenous JAs induce leaf senescence by decreasing the expression of genes involved in photosynthesis, and reducing their chlorophyll content. In Arabidopsis, senescence is correlated with increased expression of JA biosynthetic genes and increased JA levels (He et al., 2002). However JAs are not strictly required for the normal progression of
senescence as suggested by *Arabidopsis* signaling or biosynthetic mutants that do not show an obvious delayed-senescence phenotype (Howe, 2010).

Jasmonic acid seems to be involved in flower development since it is highly accumulated in reproductive tissues. In tomato flowers, jasmonic acid, MJ, OPDA and JA-Ile are accumulated in higher quantities than in leaves (Hause et al., 2000) and high levels of AOS transcripts occur (Sivasankar et al., 2000). Recently, impaired JA perception and biosynthesis mutants of tomato and *Arabidopsis* have indicated that JAs are needed for correct male and female organ development. In particular, in tomato, JAs regulate female organ and ovary development (Li et al., 2004b) while, in *Arabidopsis*, JAs seem to be essential for male reproductive growth by controlling the development of viable pollen, anther dehiscence and the elongation of anther filaments (Howe, 2010).

### 1.3.2.3 Fruit development and ripening

In apple (Fan et al., 1997a), tomato (Fan et al., 1998a), sweet cherry (Kondo et al., 2000) and grape (Kondo and Fukuda, 2001) endogenous JA levels in fruit flesh are high during intense cell division and then decrease during cell elongation. In apple and tomato, both climacteric fruits, there is another peak in endogenous JAs prior to the climacteric ethylene burst (Fan et al., 1998a) and, to a lesser extent, during ripening (Kondo et al., 2000).

The relation between ethylene and exogenously applied JAs depends on the species (Kondo et al., 2004b), the developmental stage (Fan et al., 1997b) and the applied dose (Janoudi and Flore, 2003). Exogenous post-harvest MJ treatments stimulate ethylene production in preclimacteric and climacteric fruits (Fan et al., 1998a) while it inhibits (Saniewski et al., 1987) or does not affect it in post-climacteric fruits (Fan et al., 1997b) suggesting that JA regulates the initial phases of ripening. In contrast, a pre-harvest JA treatment strongly reduces ethylene production of peach fruits at harvest (Ziosi et al., 2008a). JA mode of action seems to be linked to alteration of the activity of ACS and ACO (Fan et al., 1998a) but it can also be due to interactions with other hormones like ABA. In peach fruits, MJ treatments delay ripening by reducing transcript levels of ACO1, PG and an expansin. On the contrary in apple, exogenous JAs enhance ACO and ACS gene expression, as well as ACC and MACC content (Kondo et al., 2009), and in strawberry JAs enhances the enzymatic activity of ACS and ACO (Mukkun and Singh, 2009). It has been demonstrated that n-propyldihydrojasmonate (PDJ), a synthetic JA
derivative more effective than JA and MJ (Fujisawa et al., 1997), induces ABA accumulation in apples (Setha and Kondo, 2004) which greatly stimulates ethylene biosynthesis (Lara and Vendrell, 2000). In non-climacteric fruits, JA effect depends on application time; PDJ treatments increase sugar accumulation and color of grapes if applied at veraison, where ABA accumulation suddenly rises, but not if applied later, when ABA content decreases (Fujisawa et al., 1997; Kondo e Kawai, 1998).

JAs are also involved in fruit color and aroma development. In apples, MJ stimulates color development (Fan et al., 1998b), as well as anthocyanin accumulation (Kondo et al., 2001b) independent on fruit maturation stage. Furthermore, JAs induce chlorophyll degradation and flavonoid synthesis by stimulating chlorophyllase (CHL1; Tsuchiya et al., 1999; Wang et al., 2005), chalcone synthase (CHS; Richard et al., 2000) and phenylalanine ammoniolyase (PAL; Schenck et al., 2000) transcription, respectively. In apple, JA effects in chlorophyll degradation and anthocyanin production seem to be ethylene-independent as ethylene production is un-affected (Fan and Mattheis, 1999; Mattheis et al., 2004). In blackberries, pre-harvest MJ treatments enhances, in a dose dependent way, total anthocyanin and phenolic content, as well as flavonoid content and antioxidant activity (Wang et al., 2008). Post-harvest MJ application stimulates volatile compound (VOC) emission in pre-climacteric fruits and inhibits it in post-climacteric fruits; this effect seems to be ethylene regulated (Fan et al., 1997b).

JAs also alter other quality parameters such as flesh consistency, sugar content and acidity. In general, fruit consistency is not greatly influenced by JAs (González-Aguilar et al., 2004; Kondo et al., 2005a) while in some cases an increase in soluble solid content has been observed (Fan et al., 1998b; González-Aguilar et al., 2004; Wang et al., 2008). Pre- and postharvest JA treatment also reduces fruit susceptibility to fungi such as Penicillium digitatum in grapefruit (Droby et al., 1999), Alternaria alternata in papaya (González-Aguilar et al., 2003), Penicillium expansum and Monilia fructicola in peach and sweet cherry (Yao and Tian 2005) by stimulating fruit natural defenses, in particular the activity of chitinase, β-1,3-glucanase, PAL, peroxidase (POD) and LOX (González-Aguilar et al., 2004; Yao and Tian 2005a). Jasmonic acid and MJ can directly inhibit pathogen growth as in the case of Penicillium expansum or stimulate bio-control agents as Cryptococcus laurentii that induces defense responses in peach fruits (Yao and Tian 2005). Finally, JAs seem to be involved in the tolerance of cold damage in numerous species as demonstrated by increased oxidative stress; indeed, POD, superoxide dismutase (SOD) and alternative oxidase (AOX) transcript levels are enhanced by exogenous MJ applications (Fung et
al., 2004). Treatments with MJ and PDJ also induce a rise in ABA and polyamine content that could be correlated with cold resistance (González-Aguilar et al., 2000).

1.4 Abscisic Acid

Abscisic acid (ABA; Fig 1.4) is a well-known plant hormone, first discovered in the 1960’s under the names of either abscissin or dormin, which plays key roles in seed and organ dormancy, plant responses to biotic and abiotic stress and sugar sensing (Schwartz and Zeevaart, 2010). It belongs to a class of metabolites known as isoprenoids that are assembled from isopentenyl diphosphate (IDP) and its isomer dimethylallyl diphosphate (DMADP).

Figure 1.4 Structure of abscisic acid [(+)-S-ABA] from Schwartz and Zeevaart (2004).

1.4.1 Abscisic Acid biosynthesis and perception

ABA is synthesized in almost all cells that contain chloroplasts or amyloplasts (Taiz and Zeiger, 2010). Up to date, two distinct pathways (Fig. 1.5) for ABA biosynthesis have been proposed. A direct one that occurs in phytopathogenic fungi in which ABA is synthesized from farnesyl diphosphate (FPP), and IDP and DMADP molecules are synthesized via the well-established mevalonate (MVA) pathway (Taylor et al., 2005; Schwartz and Zeevaart, 2010). An indirect one, where ABA is produced from carotenoid cleavage, and IDP production occurs via the 2-C-methyl-d-erythritol-4-phosphate (MEP) pathway (Nambara and Marion-Poll, 2005; Schwartz and Zeevaart, 2010).
ABA biosynthesis can be divided in: a) formation of the isoprenoid precursor via MEP pathway, b) synthesis and oxidative cleavage of epoxy-carotenoids in plastids and c) transformation of xanthoxin to ABA in the cytosol (Schwartz and Zeevaart, 2010).

The MEP pathway leads to the formation of lycopene in plastids, which is used as an intermediate in the synthesis of more complex carotenoids, including xanthophylls, in photosynthetic tissues. The over-expression of the enzymes 1-deoxy-D-xylulose 5-phosphatase-synthase (DXS), hydroxymethylbutenyl 4-diphosphate reductase (HDR) and phytoene synthase (PSY) often results in increased ABA levels (Taylor et al., 2005). Subsequently, lycopene is cyclized and hydroxylated to form zeaxanthin via the β-branch of carotenoid synthesis (Nambara and Marion-Poll, 2005; Taylor et al., 2005).

The epoxycarotenoid cleavage is the first committed step of ABA biosynthesis and is catalyzed by 9-cis-epoxycarotenoid dehydrogenase (NCED), which cleaves 9-cis-violaxanthin and 9-cis-neoxanthin onto 9-cis-xanthoxin (Schwartz and Zeevaart, 2010). An impaired maize mutant for this step showed a 35% and 70% reduction in ABA accumulation in stressed leaves.
and developing embryos, respectively (Tan et al., 1997). NCED are encoded by a family of genes located on the stromal face of thylakoids in soluble and membrane-bound forms. Their expression patterns vary in response to stress and developmental signals (Taiz and Zeiger, 2010). Tomato mutants over-expressing NCED show enhanced ABA accumulation in leaves, root and xylem sap (Thompson et al., 2007). In bean, stress-induced ABA biosynthesis correlates well with increased expression of NCED mRNA and with NCED protein levels; the stress alleviation reduces PvNCED1 expression and ABA levels. In avocado fruits, PaNCED1 and PaNCED3 transcript levels increase prior to the accumulation of ABA during ripening thus suggesting that NCED is a major rate-limiting step in ABA biosynthesis (Schwartz and Zeevaart, 2010).

The newly formed 9-cis-xanthoxin exits the chloroplast and is converted to ABA by cytoplasmic enzymes. This process consists of two steps: first the formation of abscisic aldehyde which is regulated by a short-chain alcohol dehydrogenase (ABA2) encoded by the ABA2 locus in Arabidopsis, and then the formation of ABA by the action of an abscisic aldehyde oxidase which requires a molybdenum cofactor (AAO3; Nambara and Marion-Poll, 2005; Taiz and Zeiger, 2010). AAO mutants lacking a functional molybdenum cofactor, as Arabidopsis aba3 and tomato flacca, are unable to synthesize ABA (Taiz and Zeiger, 2010). The aba2 Arabidopsis mutant has impaired conversion of xanthoxin to abscisic aldehyde, and shows sugar-insensitive phenotype and altered stomatal conductance (Schwartz and Zeevaart, 2010).

Once ABA is formed, it can be rapidly metabolized in situ or it can leave the cell and functions as a long distance signal through the plant. ABA catabolism rate is proportional to the amount of ABA accumulated since there are no substantial differences in ABA half-life in maize under well-watered and stress conditions (Ren et al., 2007). The catabolism mainly occurs by two types of reactions; a) hydroxylation of the 8’ position by the enzyme ABA 8’-hydroxylase (Fig. 1.6) which mainly produces phaseic acid (PA) and dihydrophaseic acid (DPA); and b) conjugation of the carboxyl group to glucose by the enzyme ABA glucosyl transferase to convert it to ABA glucosyl ester (ABA-GE). Both reactions seem to be induced by ABA since an increase in ABA to PA conversion occurs in suspension cultures pre-treated with ABA, and the accumulation of ABA-GE increases in vacuoles of plants subjected to a series of stress and re-watering cycles (Schwartz and Zeevaart, 2010). The catabolism produces an inactivation of ABA; however, ABA-GE seems to be important in the long distance transport of ABA as its concentration is high in xylem, and remains stable from root to shoot because it has low
permeability. In contrast, free ABA is compartmentalized following the “anion trap” mechanism (Jiang and Hartung, 2008; Dodd and Davies, 2010; Taiz and Zeiger, 2010).

![Figure 1.6. Schematic representation of ABA catabolism due to hydroxylation reactions (Nambara and Marion-Poll, 2005).](image)

Recently, several putative ABA receptors have been identified and proposed. They include: the PYR/PYL/RCAR class of soluble receptors, members of the START superfamily of ligand-binding proteins in the cytosol and nucleus (Taiz and Zeiger, 2010); CHLH, which is a plastid-localized subunit of the Mg-cheletase enzyme involved in chlorophyll synthesis, stomatal signaling, and seed development and post-germination growth (Shen et al., 2006); FCA, flowering time control protein A, a nuclear receptor involved in regulation of ABA-dependent flower transition and also in responses of lateral root growth to ABA (Razem et al., 2006); GCR2, part of the G protein coupled receptors, that controls major ABA responses such as seed germination and dormancy (Liu et al., 2007).

1.4.2 Physiological roles of ABA

1.4.2.1 Stress responses and stomata control

The most studied role of ABA concern its involvement in plant stress responses, mainly water and osmotic stresses, and stomata control. Drought is one of the mayor environmental stresses
that limit crop growth and productivity (Iuchi et al., 2001). Cell expansion and growth are among the first processes to decline under water deficit, while progressive water deficit negatively affects photosynthesis and carbon partitioning (Chaves et al., 2002; Taiz and Zeiger, 2010). From a cellular point of view, membranes and proteins can be damaged by reduced hydration and can lead to increases in reactive oxygen species (Artlip and Wisnieski, 2002).

The turgor pressure of guard cells, which responds to environmental and physiological signals, modulates stomatal aperture and thereby regulates leaf water loss and carbon dioxide assimilation (Brodribb and McAdam, 2011). Stomatal opening and closure are osmotically-driven processes that occur by changes in guard cell turgor pressure; guard cell swelling widens the pore and guard cell deflating narrows it. Stomatal opening is mainly due to the activation of plasma-membrane H\(^+\) ATPases that extrude protons from guard cells and create a membrane hyperpolarization that generates a driving force for K\(^+\) uptake, along with Cl\(^-\) uptake and malate\(^2-\) synthesis in the cytosol, inducing water influx and cell swell (Assmann, 2010; Taiz and Zeiger, 2010). It has been demonstrated that ABA produces membrane depolarization by reducing K\(^+\) influx, inducing Cl\(^-\) and malate\(^2-\) efflux and increasing cytosolic calcium concentration of guard cells, as well as it inhibits H\(^+\) ATPase activation by blue light so that water is osmotically lost and stomata close (Taiz and Zeiger, 2010).

Stressful conditions stimulate ABA synthesis in plants and detached tissues (Cornish ans Zeevaart, 1985; Zeevaart and Creelman, 1988; Dodd and Davies, 2010). This increase in ABA levels can reach 200- to 3000-fold in xylem sap and 50-fold in leaves within 4 to 8 hours in water-stressed sunflower and maize plants (Taiz and Zeiger, 2010). Leaf ABA increment leads to stomatal closure, widespread changes in gene expression and other adaptations that increase plant stress tolerance (Schwartz and Zeevaart, 2010).

Since NCED is thought to function in the rate limiting step of ABA biosynthesis, drought-inducible NCED may be responsible for the accumulation of ABA under stress conditions, and thus for the induction of stomatal closure. Drought-inducible NCED genes have been reported in Arabidopsis (Iuchi et al., 2001), maize (Schwartz et al., 1997), tomato (Thompson et al., 2007) and tobacco (Qin and Zeevaart, 2002). In drought-stressed Arabidopsis, the expression of AtNCED3 is strongly induced, and altering AtNCED3 expression in transgenic plants affects endogenous ABA levels, stomata closure and plant tolerance to drought (Iuchi et al., 2001). Tomato mutants over-expressing LeNCED1 present enhanced stomatal response to vapor
pressure deficit (VPD) than wild type under well-watered conditions thus exhibiting improved transpiration efficiency and soil water conservation (Thompson et al., 2007).

Exogenous ABA mimics the effect of water stress by causing stomata closure, increasing leaf water potential and reducing transpiration (Quarrie and Jones, 1977). In tobacco plants, stress conditions lead to a concomitant increase in ABA and PA levels, while DPA remains low, suggesting that also ABA catabolism is enhanced (Qin and Zeevart, 2002). Application of exogenous ABA to ABA-deficient mutants of maize causes stomatal closure and restoration of turgor pressure while *wilty* mutants blocked in their ability to respond to ABA are not rescued by ABA application (Taiz and Zeiger, 2010).

Salinity stress is related with water deficit since the excess of ions in the system decreases plant water status. As well as in water stress, many proteins and gene transcripts increase in response to salinity stress, and many of them are inducible by ABA (Artlip and Wisniewski, 2002). The induction of osmotic stress and/or exogenous ABA treatments to bean plants increase ABA content in leaves, roots and nodules (Khadri et al., 2006). In citrus (Gómez-Cardenas et al., 2003; Arbona et al., 2006) and beans (Khadri et al., 2006) plant tolerance to osmotic stress is enhanced by exogenous ABA treatments since they reduce leaf ethylene production and leaf abscission in citrus, and increase plant growth in beans. The induction of osmotic stress in *Arabidopsis* confirms that *NCED3* and other biosynthetic ABA genes are induced by salinity stresses which positively regulate ABA biosynthesis (Barrero et al., 2006).

Regarding biotic stress, ABA is commonly associated with inducing pathogen susceptibility as demonstrated by ABA-deficient mutants in which pathogen resistance increases 20- to 80-fold as compared to wild-type plants (Wasilewska et al., 2008). ABA mainly influences disease resistance by interfering with biotic stress signaling mediated by SA, jasmonic acid and ethylene (Fig. 1.7; Mauch-Mani and Mauch, 2005). Also, disruption of ABA signaling induces the expression of JA and ethylene responsive genes (Mauch-Mani and Mauch, 2005). In some cases ABA seems to positively influence biotic stress responses by increasing callose deposition through the enhancement of diverse callose synthase activities, especially PMR4 (Mauch-Mani and Mauch, 2005). Moreover, *Arabidopsis pmr4-1* mutant deficient in callose deposition, and wild-type plants treated with exogenous ABA present diminished *Alternaria brassicicola* infection (Flors et al., 2008).
1.4.2.2 Seed development

Typically, seed ABA content is very low early in embryogenesis, reaches a maximum at about halfway point, and then gradually falls to low levels as the seed reaches maturity. Thus, there is a broad peak of ABA accumulation in the seed corresponding to mid- to late embryogenesis (Taiz and Zeiger, 2010) which correlates with the cessation of cell divisions in the embryo (Finkelstein, 2010). Seed maturation period is characterized by massive reserve accumulation and cell enlargement, as cells get filled with protein and lipid bodies that will support growth at germination (Kinkelstein, 2010). In this phase, ABA stimulates the accumulation of late-embryogenesis (LEA) proteins that are thought to confer seed desiccation tolerance; in fact, exogenous ABA treatments enhance the accumulation of storage proteins and LEA proteins in cultured embryos, and ABA-deficient and insensitive mutants fail to accumulate them (Kinkelstein, 2010; Taiz and Zeiger, 2010).

Also, ABA suppresses germination of mature seeds and vivipary (Kinkelstein, 2010). Immature embryos placed in a medium with or without added ABA, germinate precociously or do not germinate, respectively. In maize, several mutants that show vivipary are ABA-deficient and this can be partially prevented by exogenous ABA treatments (Taiz and Zeiger, 2010). Finally, ABA plays a crucial role in seed dormancy since ABA-deficient and insensitive
*Arabidopsis* mutants are non-dormant at fruit ripening or show reduced dormancy, respectively (Taiz and Zeiger, 2010), and ABA hypersensitive mutants present increased dormancy suggesting that ABA is an important endogenous inhibitor of germination (Kinkelstein, 2010).

1.4.2.3 Plant development

ABA was the first inhibitory hormone known to be involved in the regulation of growth together with growth promoters. At concentrations of $10^{-7}$ to $10^{-5} \text{ M}$ ABA inhibits growth of wheat coleptiles, barley shoots, bean axes, and the second leaf sheaths of rice seedlings (Naqvi, 2002). In contrast, recent studies suggest that ABA is needed for normal plant growth and upon growth conditions it can inhibit or promote growth (Sharp *et al.*, 2002; LeNoble and Sharp, 2004). At high water potential shoot growth, and root growth to a lesser extent, is greater in wild-type maize plants than in ABA-deficient mutants. In contrast, limiting water availability determines opposite effects on shoots and roots, with shoot growth being greater in ABA-deficient mutants and root growth in wild-type plants (Sharp *et al.*, 2002). In ABA-deficient *flacca* and *notabilis* mutants of tomato, normal levels of ABA are required to maintain shoot development in well-watered plants (Sharp *et al.*, 2000). Exogenous ABA treatments inhibit plant growth under normal growth conditions and enhance it under osmotic stress in common bean (Khadri *et al.*, 2006). Under high osmotic conditions, ABA-deficient mutants of *Arabidopsis* have an increased root system as compared to wild-type plants (De Smet *et al.*, 2006) thus indicating that ABA may inhibit growth under non-stressed conditions and enhance it under stressful ones; however this mutants show abnormal growth in both non-stressed and stressed conditions. The resulting growth inhibition by ABA deficiency is correlated with overproduction of ethylene; augmenting the endogenous ABA content by exogenous treatments resumes plant growth by inhibiting ethylene production (Sharp *et al.*, 2000; Sharp *et al.*, 2002);

ABA is also involved in root branching since ABA-deficient mutants of *Arabidopsis* show a decrease in the number of lateral roots formed as compared to wild-type plants (De Smet *et al.*, 2006). Moreover, insensitive ABA mutants exhibit decreased sensitivity to auxin-induced lateral root initiation (Nibau *et al.*, 2008) and present reduced branching inhibition by high-nitrate conditions (Taiz and Zeiger, 2010). In rice, on the contrary, ABA seems to stimulate lateral root initiation and elongation since ABA-deficient mutants show inhibited root formation and elongation (Chen *et al.*, 2006).
1.4.2.4 Fruit development and ripening

ABA can be considered as a ripening control factor, because its content is low during fruit
development and increases towards ripening. In non-climacteric fruits such as citrus (Richardson
and Cowan, 1995), grape (Kondo and Kawai, 1998), litchi (Wang et al., 2007), rambutan (Kondo
et al., 2001a) and sweet cherry (Kondo and Gemma, 1993) ABA concentration peaks before
maturation and then decreases towards harvest. In climacteric fruits, such as apple (Lara and
Vendrell, 2000), peach (Zhang et al., 2009a) and tomato (Zhang et al., 2009b), ABA
accumulation precedes ethylene production or occurs concomitantly with rises in ACC and
MACC content.

Also, ABA is well correlated with ripening-related changes such as color development, sugar
and anthocyanin accumulation, softening progression and chlorophyll degradation. Several
studies demonstrated that exogenously applied ABA accelerates ripening of diverse species by
enhancing color development (Kondo and Gemma, 1993; Jiang and Joyce, 2003; Cantín et al.,
2007; Peppi et al., 2007; Wang et al., 2007; Peppi et al., 2008), anthocyanin accumulation
(Kondo and Gemma, 1993; Kondo and Kawai, 1998; Jeong et al., 2004; Wang et al., 2005; Peppi
et al., 2006; Peppi et al., 2007; Wang et al., 2007; Peppi et al. 2008), softening (Jiang and Joyce,
2003; Peppi et al., 2007), sugar accumulation (Kojima et al., 1995; Ofosu-Anim, 1996; Kondo
and Kawai, 1998; Ofosu-Anim, 1998; Kobashi et al., 2001; Jeong et al., 2004), chlorophyll
degradation (Wang et al., 2005) and decreasing titratable acidity (Peppi et al., 2007). In
climacteric fruits this ripening acceleration is attributed to increased ethylene production (Bruesa
and Vendrell, 1989; Jiang and Joyce, 2003).

In tomato, peach and grape, endogenous ABA accumulation occurs before ethylene
production (Zhang et al., 2009 a and b), indicating that endogenous ABA is critical for the onset
of ripening. In climacteric and non-climacteric fruits, ABA accumulation during ripening occurs
after the increase in transcript levels of ABA biosynthetic enzymes NCED (LeNCED1,
PpNCED1 and VvNCED1 in tomato, peach and grape respectively) suggesting that NCED over-
expression initiated ABA biosynthesis at the beginning of fruit ripening. In both studies,
exogenous ABA accelerated fruit ripening while treatments with fluridone, an inhibitor of
carotenoid synthesis and thereby of ABA synthesis (Sharp et al., 2002), and 1-MCP delayed
ripening. In contrast, 1-MCP treatments did not inhibit ABA synthesis and enhanced ABA
accumulation in peach and grape. Reduction of ABA content effectively postponed maturity and
softening suggesting that ABA also plays a role in later stages of ripening in both climacteric and non-climacteric fruits (Zhang et al., 2009a and b).

Color development and anthocyanin accumulation by ABA correlate with enhanced key regulatory steps of anthocyanin biosynthesis such as PAL activity in strawberry (Jiang and Joyce, 2003) and gene expression of UDP glucose:flavonoid 3-O-glycoltransferase (UFGT) (Jeong et al., 2004; Peppi et al., 2008) chalcone isomerase (CHI) and CHS in grapes (Jeong et al., 2004). Chlorophyll degradation in litchi, instead, correlates with an increasing activity of chlorophyllase (Wang et al., 2005).

Regarding sugar metabolism, exogenous ABA stimulates sink activity and assimilate unloading as demonstrated in fruit discs by the stimulation of passive sugar uptake in melon (Ofosu-anim et al., 1998) and strawberry (Ofosu-Anim et al., 1996), and active sugar uptake in peach (Kobashi et al., 2001). Also, ABA treatments modify the activity of sugar metabolizing enzymes; in peach ABA transiently enhances the activity of sorbitol oxidase (SOX; Kobashi et al., 1999); in grape and apple it induces both soluble (SAI) and cell wall acid invertases (CWAI; Pan et al., 2005; Pan et al., 2006) and activates ATPase in apple fruits, thus enhancing energy-driven carbohydrate unloading (Peng et al., 2003).

### 1.5 Aims of the Thesis

The aim of the present study/thesis was to better understand the physiological role of the phytohormones JAs and ABA in fruit ripening. In particular, the effects of exogenous application of these substances at different developmental stages and under different experimental conditions were evaluated at agronomical, physiological and molecular levels. Three fruit species, peach, golden and green kiwifruit, and several cvs were used for the trials. Different experimental models were adopted: fruits in planta, detached fruit, detached branches with fruit, girdled branches and micropropagated plants. Agronomical and molecular analyses were performed in order to get a deeper insight into the physiological changes induced by the hormones in prospect of a possible field application of JAs and ABA to improve fruit yield and quality. In particular, JA/fruit quality, JA/ethylene, JA/auxin and ABA/fruit quality relationships were investigated in peach and kiwifruit. Moreover, some aspects of the ABA/water relationships were analyzed in both species. The work was structured into four sets of experiments as follows:
(i) Pre-harvest MJ application was performed at S3/S4 transition under field conditions in Redhaven peach; ethylene production, ripening index, fruit quality and shelf-life were assessed; moreover, the time course of the expression of ethylene-, auxin- and other ripening-related genes was determined.

(ii) Pre- and post-harvest ABA applications were carried out under field conditions in Flaminia and O’Henry peach and Stark Red Gold nectarine fruit at different developmental stages; ethylene production, ripening index, fruit quality and shelf-life were assessed together with tree water potential.

(iii) Pre- and post-harvest ABA and PDJ treatments were carried out under field conditions at different fruit developmental stages and in post-harvest in kiwifruit. Ripening index, fruit quality, plant transpiration, photosynthesis and stomatal conductance were assessed.

(iv) Spray, drench and dipping ABA treatments were performed in micropropagated peach plants and in peach and nectarine detached branches; plant water use and transpiration, biomass production and fruit dehydration were determined.
2. PRE-HARVEST METHYL JASMONATE APPLICATION DELAYS FRUIT RIPENING AND ALTERS ETHYLENE AND AUXIN BIOSYNTHESIS AND PERCEPTION IN PEACH

2.1 Introduction

Jasmonates (JAs) are ubiquitous signaling molecules, synthesized from linolenic acid, which mediate plant responses to environmental stress such as wounding and pathogen and insect attacks (Wasternack, 2007).

JAs also play a role during developmental processes, including plant growth, seed germination, pollen development, and fruit development and ripening (Peña-Cortés et al., 2005; Wasternak, 2007). Ripening is a complex, genetically programed process: in climacteric fruit, progressive physicochemical changes involving color, texture, flavor and aroma, which all contribute to overall fruit quality, are induced and, at least in part, co-ordinated by changes in ethylene biosynthesis and perception (Giovannoni, 2004). In climacteric fruit, such as apple and tomato, JA levels increase at ripening suggesting that they could be involved in the modulation of this process (Fan et al., 1998a; Kondo et al., 2000). Exogenously applied methyl jasmonate (MJ) was shown to inhibit or enhance fruit ethylene production in apples and pears in relation with fruit ripening stage (Fan et al., 1997b; Kondo et al., 2007). In nectarines, pre-harvest JA treatment resulted in delayed ripening as determined by lower ethylene production rates, reduced fruit softening and down-regulation of several ripening-related genes (Ziosi et al., 2008a; Ziosi et al., 2009); in peaches, post-harvest MJ application reduced fruit softening and flesh browning during cold storage (Meng et al., 2009).

In the present work, peach was chosen as a model to shed some light on the physiological role of JAs during fruit ripening and shelf-life, and the reciprocal relationship between ethylene and JAs. Moreover, the effects of JAs on the expression of auxin-related and other ripening-related genes were evaluated in order to better understand the fruit physiological response. The natural derivative of jasmonic acid, MJ, was applied to peach fruit at S3/S4 developmental stage under field conditions, and the following were analyzed: (i) ethylene production, fruit ripening and quality; (ii) transcript levels of several ethylene-, auxin- and ripening-related genes (iii) and postharvest behavior.
2.2 Materials and Methods

2.2.1 Plant material and experimental design

The trial was conducted at the S. Anna experimental field of the University of Bologna, Italy, on twenty year old peach trees (*Prunus persica* L. Batsch), cv. RedHaven, grafted on seedling rootstock and trained to a free open-vase. Eight trees were randomly selected for their size and fruit-load uniformity; half of them were sprayed with 200 ppm MJ (Nippon Zeon Co., Tokyo, Japan) aqueous solution; the latter was prepared by diluting a 5% MJ stock solution containing 30% (v/v) surfactant (Rheodor460, Nippon Zeon Co., Tokyo, Japan) and 32.5% (v/v) ethanol (Ziosi et al., 2008a); the remaining four plants were sprayed with an aqueous solution containing the same concentration of surfactant and ethanol. Treatments were performed at the S3/S4 transition stage of fruit growth. Fruits (100-150) were picked 11 and 14 days after treatment (DAT); a part of them (20 fruits selected by the DA-meter) was left at room temperature (20°C) in order to assess their ethylene production after 0, 12, 36 and 60 h of shelf-life; the remaining fruits were used for non-destructive ripening assessments. Also at 2 and 7 DAT destructive and non-destructive quality analysis, as well as ethylene production determination were performed to a 20fruit sample (20). Fruit mesocarp tissue was sampled for molecular analysis and phenolic determination at 2, 7, 11 and 14 DAT, and stored at -80°C until use.

2.2.2 Ethylene and quality trait determinations

At harvest, the extent of fruit ripening was non-destructively measured by means of the DA-meter, a portable and non-destructive device based on visible/Near Infra-Red (Vis/NIR) spectroscopy developed and patented by the University of Bologna (Costa et al., 2005). This instrument gives a fruit maturity index, called “Index of Absorbance Difference” (I<sub>AD</sub>) that is based on fruit absorbance spectra acquired by a spectrometer in the 650-1200 nm wavelength range and is calculated as:

\[ I_{AD} = A_{670} - A_{720} \]

where \( A_{670} \) and \( A_{720} \) are the absorbance values at the wavelengths of 670 and 720 nm, respectively. This difference in absorbance between two wavelengths near the chlorophyll-a peak
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

(I\textsubscript{AD}) is strictly correlated to the actual chlorophyll-a content in peach fruit flesh and to the time course of ethylene production during fruit ripening (Ziosi et al., 2008b). Therefore, considering that peach is a climacteric fruit whose chlorophyll content decreases during ripening (Chalmers and Ende, 1975), the I\textsubscript{AD} allows to group peach fruit on the basis of their ripening stage in homogeneous classes. In particular, the I\textsubscript{AD} continuously decreases during the progression of peach fruit ripening (Ziosi et al., 2008b).

Ethylene production was measured by placing the whole detached fruit in a 1.0 l jar with an air-tight lid equipped with a rubber stopper, and left at room temperature for 1 h. A 10 ml gas sample was taken and injected into a Dani HT 86.01 (Dani, Milan, Italy) gas chromatograph fitted with a flame ionization detector and a Porapak Q column (Supelco, Bellefonte, PA, USA). The carrier gas was nitrogen at a flow rate of 16 ml min\textsuperscript{-1}. The oven temperature was 80ºC for the column and 150ºC for the injector and flame ionization detector. Ethylene was identified and its concentration was calculated on the basis of the following standards (SIAD, Bologna, Italy): 3.5 ppm C\textsubscript{2}H\textsubscript{4} and 5150 ppm CO\textsubscript{2}, 20.81% O\textsubscript{2} in N\textsubscript{2}. (Bregoli et al., 2002).

Flesh firmness (FF) was measured using a pressure tester (EFFE.GI, Ravenna, Italy), fitted with an 8 mm plunger on the two opposite cheeks of each fruit, after removing a thin layer of epicarp; soluble solids concentration (SSC) was determined with an Atago digital refractometer (Optolab, Modena, Italy) by squeezing part of the mesocarp; titratable acidity (TA) was determined on 20 ml of flesh juice (titration with 0.25 N NaOH) using a semiautomatic instrument (Compact-S Titrator, Crison, Modena, Italy).

2.2.3 Phenolic extraction and determination

Phenolic determinations were done following a protocol described previously by Andreotti et al. (2008). Briefly, frozen mesocarp samples were lyophilized and ground to a fine homogeneous powder which was then extracted with 1 ml methanol containing 6-methoxy-flavone (0.025 mg ml\textsuperscript{-1} in methanol) as an internal standard.

Phenolic extracts were analysed by a Waters HPLC (Waters corp., Milford, MA, USA) system with a Photodiode Array Detector (Waters 2996) and a reverse-phase Supelcosil\textsuperscript{TM} LC-18 HPLC column (15 cm long, 4 mm internal diameter containing octadecyl silane particles of 5 μm diameter); following the methodology described by Andreotti et al. (2008).
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

Phenolic compound identification was carried out through a comparison of retention time values and UV spectra (detected between 210 and 560 nm wavelength) with authentic standards. Phenolic compound concentrations, expressed in mg g\(^{-1}\) dry weight (DW), were calculated from calibration curves obtained with the corresponding external standards.

Standards for qualitative and quantitative determinations were purchased from Sigma-Aldrich (St Louis, MO, USA) whereas methanol, for liquid chromatography, and phosphoric acid were purchased from Carlo Erba (Milan, Italy).

2.2.4 Gene expression analysis by Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from mesocarp samples according to Chang et al. (1993). RNA yield and purity were checked by means of UV absorption spectra, whereas RNA integrity was determined by electrophoresis in agarose gel. DNA was removed from 10 µg aliquots of total RNA using the TURBO DNA-free™ (Applied Biosystems, Foster City, CA, USA). The first-strand cDNA was synthesized from 3 µg of the DNaseI-treated RNA by means of the High-Capacity cDNA Kit (Applied Biosystems), using random primers.

Real-time RT-PCR was performed in a reaction mixture, final volume 25 µl, containing 9 ng of cDNA, 5 pmol of each primer, and 12.5 µl of the Fast SYBR Green PCR master mix (Applied Biosystems), according to the manufacturer’s instructions. The oligonucleotides DZ79 (5’-TGACCTGGGGTTCGCGTTGAA-3’, sense) and DZ81 (5’-TGAATTGCAGAATCCCGTGAA-3’ antisense), annealing to the Internal Transcribed Spacer (ITS) of the rRNA, were used to amplify the internal standard with peach samples. The ethylene and auxin-related, and AOS, NCED and bZIP primer sequences (Trainotti et al., 2007) are listed in table 2.1.
**Table 2.1** Sequences of oligonucleotides used in real-time qRT-PCR expression analysis.

<table>
<thead>
<tr>
<th>Contig name</th>
<th>Oligo name</th>
<th>Sequence of oligo</th>
<th>Contig annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>ctg_64_for</td>
<td>CCCCCATGCAGCCACTCCA</td>
<td>1- Aminocyclopropane-1-carboxylate oxidase (ACO1)</td>
</tr>
<tr>
<td></td>
<td>ctg_64_rev</td>
<td>CATCAGCTGCCAGGTGTAAG</td>
<td></td>
</tr>
<tr>
<td>298</td>
<td>ctg_298_for</td>
<td>TGTTCCCCTTGTACCTTGCTGTC</td>
<td>bZIP transcription factor</td>
</tr>
<tr>
<td></td>
<td>ctg_298_rev</td>
<td>AAGATGGGTTTGGGATTTGA</td>
<td></td>
</tr>
<tr>
<td>475</td>
<td>ctg_475_for</td>
<td>TGTTCCCCTTGTACCTTGCTGTC</td>
<td>IAA-amino acid hydrolase</td>
</tr>
<tr>
<td></td>
<td>ctg_475_rev</td>
<td>AAGATGGGTTTGGGATTTGA</td>
<td></td>
</tr>
<tr>
<td>489</td>
<td>ctg_489_for</td>
<td>TGTTCCCCTTGTACCTTGCTGTC</td>
<td>1-Aminocyclopropane-1-carboxylate synthase (ACS1)</td>
</tr>
<tr>
<td></td>
<td>ctg_489_rev</td>
<td>TCTTTGCCGCGATGTCACC</td>
<td></td>
</tr>
<tr>
<td>1436</td>
<td>ctg_1436_for</td>
<td>AAGGCATAGCCTGGATGCTTGTA</td>
<td>Ethylene receptor (ETR-1)</td>
</tr>
<tr>
<td></td>
<td>ctg_1436_rev</td>
<td>TCCCTTGCTCCTTATGGCTTCT</td>
<td></td>
</tr>
<tr>
<td>1993</td>
<td>ctg_1993_for</td>
<td>AAGAGCGGACGTTTTGAGGAGTT</td>
<td>Auxin-responsive GH3 family protein</td>
</tr>
<tr>
<td></td>
<td>ctg_1993_rev</td>
<td>CAGTGCGGTAAGATGGGCTAAAA</td>
<td></td>
</tr>
<tr>
<td>2116</td>
<td>ctg_2116_for</td>
<td>AGGGGTTCAGGTGGGTGCTTGTA</td>
<td>Ethylene response factor 2</td>
</tr>
<tr>
<td></td>
<td>ctg_2116_rev</td>
<td>GCTTTGGGTTGGATGTCGTC</td>
<td></td>
</tr>
<tr>
<td>2713</td>
<td>ctg_2713_for</td>
<td>GGGTGACTGAATCTGGGTTTG</td>
<td>Transport inhibitor response 1 (TIR1)</td>
</tr>
<tr>
<td></td>
<td>ctg_2713_rev</td>
<td>TGTTGGCCTTGGGCTTAT</td>
<td></td>
</tr>
<tr>
<td>2980</td>
<td>ctg_2980_for</td>
<td>GGACGAGGGGTACATAATGACTTT</td>
<td>9-cis-epoxycarotenoid dioxygenase (NCED)</td>
</tr>
<tr>
<td></td>
<td>ctg_2980_rev</td>
<td>CGGCAGGAGCCCTTGATGG</td>
<td></td>
</tr>
<tr>
<td>3371</td>
<td>ctg_3371_for</td>
<td>AAGTGCAAGCCTGGATTACC</td>
<td>Tryptophan synthase Ϝ subunit (Wsynt)</td>
</tr>
<tr>
<td></td>
<td>ctg_3371_rev</td>
<td>TAGGCCAGCTGATGAGAAGTC</td>
<td></td>
</tr>
<tr>
<td>3575</td>
<td>ctg_3575_for</td>
<td>ACAACCGCAATCTGGGAAACAT</td>
<td>Indol-3-glycerol-phosphate synthase (IGPS)</td>
</tr>
<tr>
<td></td>
<td>ctg_3575_rev</td>
<td>TAGGCAATATCATCAGGAGTCG</td>
<td></td>
</tr>
<tr>
<td>3721</td>
<td>ctg_3721_for</td>
<td>ATGATGGCGGCTGGGAAGGAAT</td>
<td>PIN1-like auxin transport protein</td>
</tr>
<tr>
<td></td>
<td>ctg_3721_rev</td>
<td>TGCTGTGCGCCGCGGTAAT</td>
<td></td>
</tr>
<tr>
<td>AOS F</td>
<td>n/a</td>
<td>GAGCTCACAGGAGGTTACAG</td>
<td>AOS (Homemade)</td>
</tr>
<tr>
<td>AOS R</td>
<td>n/a</td>
<td>CGAGATGGAACTCGGGTAG</td>
<td></td>
</tr>
</tbody>
</table>
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

PCRs were carried out with the StepOnePlus™ 7500 Fast (Applied Biosystems) for 2 min at 95 °C and then for 40 cycles as follows: 95 °C for 15 s, 60 °C for 15 s, and 65 °C for 34 s. The obtained CT values were analyzed with the Q-gene software by averaging three independently calculated normalized expression values for each sample. Expression values are given as the mean of the normalized expression values of the triplicates, calculated according to equation 2 of the Q-gene software (Muller et al., 2002).

2.2.5 Statistical analysis

All data were statistically analyzed using a completely randomized design. The treatment was the only factor (2 levels: MJ and control) for the majority of the analyzed parameters. For ethylene production during shelf-life the factors were the treatment (2 levels: MJ and control) and the ripening class selected (2 levels: climacteric and non-climacteric); when significant interaction occurred, the treatment factor was analyzed separately per each level of ripening class. Mean separation analysis was performed by the Student Newman-Keuls test.

2.3 Results and Discussion

2.3.1 Exogenous MJ delays peach ripening

Destructive quality evaluations, carried out 2 and 7 days after MJ treatments (Tables 2.2 and 2.3), revealed that treated fruits had higher FF than controls at the latter evaluation date while no significant differences in SSC and ethylene were observed at either determination date.

**Table 2.2** Effect of MJ treatments on main fruit quality parameters 2 days after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (ºBrix)</th>
<th>Ethylene (nl g(^{-1}) FW h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>7.46 a</td>
<td>10.53 a</td>
<td>0.0000 a</td>
</tr>
<tr>
<td>Control</td>
<td>7.81 a</td>
<td>10.81 a</td>
<td>0.0000 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

Table 2.3 Effect of MJ treatments on main fruit quality parameters 7 days after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm(^{-3}))</th>
<th>SS (°Brix)</th>
<th>Ethylene (nl g(^{-1}) FW h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>6.54 a</td>
<td>10.43 a</td>
<td>0.0005 a</td>
</tr>
<tr>
<td>Control</td>
<td>5.53 b</td>
<td>10.80 a</td>
<td>0.0000 a</td>
</tr>
</tbody>
</table>

**Significance**

n.s., not significant; **, significant difference at P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Non-destructive (Table 2.4) evaluations showed that mean fruit I\(_{AD}\) exhibited a decreasing trend during the considered period in both treated and untreated fruits; from 7 DAT on, mean I\(_{AD}\) was significantly higher in treated fruits as related to control ones.

Table 2.4 Effect of MJ treatments on fruit maturation (I\(_{AD}\)) measured with the DA-meter.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Treated</td>
<td>1.584 a</td>
</tr>
<tr>
<td>Control</td>
<td>1.570 a</td>
</tr>
</tbody>
</table>

**Significance**

n.s., not significant; *, significant difference at P ≤ 0.05; **, P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Diverse results have been reported concerning JA effects on ripening-related parameters; in fact, whereas anthocyanin accumulation is generally stimulated in JA-treated fruit (Rudell et al., 2002; Rudell et al., 2005), other ripening related parameters such as fruit FF and SSC may be unaltered or differentially affected (Gonzalez-Aguilar et al., 2004; Kondo et al., 2005; Ziosi et al., 2008a). In nectarines, MJ and PDJ field applications reduced ethylene emission, softening and color development as related to the application time (Ziosi et al., 2008a). During peach and nectarine ripening progression, fruit I\(_{AD}\) values drops abruptly along with the rise in climacteric ethylene production and the decrease of chlorophyll content in outer mesocarp of Stark Red Gold nectarines (Ziosi et al., 2008b). Thereby, the observed reduction in softening and the delay in I\(_{AD}\) progression confirm that MJ induces a ripening delay in peach fruit.
2.3.2 Exogenous MJ modifies flesh phenolic composition without altering total content

The phenolic compounds detected in flesh of treated and untreated ‘RedHaven’ fruits belonged to two main classes, cinnamic acids and flavan-3-ols; moreover, unidentified compounds were classified as unknown. Cinnamic acid and flavan-3-ol content in treated and control fruits decreased during development and ripening. In treated fruit, phenolic determinations (Fig. 2.1) showed a significant increase of unknown compound content at 2 and 7 DAT without significant alterations of cinnamic acids, flavan-3-ols or total phenolic content even though they all tended to be lower in treated fruits at 11 and 14 DAT.

![Figure 2.1](image)

**Figure 2.1** Effect of MJ treatments on flesh anthocyanin composition. a, total phenols; b, cinnamic acids; c, flavan-3-ols; d, unknown. **, significant difference at P ≤ 0.01. Bars indicate mean ± standard error.

The present results show that peach flesh phenolic composition is not significantly affected by MJ treatments. In contrast, MJ treatment stimulates anthocyanin biosynthesis in apples
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

(Kondo et al., 2001; Rudell et al., 2002; Rudell and Mattheis, 2008), peach shoots (Saniewski et al., 1998a) and tulip leaves (Saniewski et al., 1998b), enhance peach skin color formation (Janoudi and Flore, 2003) and reduce flesh phenolic content of peaches during cold storage (Meng et al., 2009). It should be considered that the main phenolic compound accumulation in peach fruit occurs in the skin while flesh accumulates phenolics only at early stages of development and their concentration decreases during development (Andreotti et al., 2008).

2.3.3 Exogenous MJ alters the expression pattern of ethylene-related genes

Transcription pattern of ethylene related genes was differentially affected by MJ-treatments. In control fruit, transcript levels of ethylene biosynthetic genes (ACO1 and ACS1; Fig 2.2) showed an increasing trend from 0 to 14 DAT; in MJ-treated fruit, ACO1 expression was transiently inhibited according to the higher IAD values while ACS1 transcript accumulation was initially inhibited and then enhanced; this could be due to a recovery in ethylene synthesis which follows MJ-induced inhibition.

**Figure 2.2** Effect of MJ treatments on ethylene biosynthetic genes transcript levels. a, ACO1; b, ACS1. ***, significant difference at P ≤ 0.01; ***, P ≤ 0.001. Bars indicate mean ± standard error.

Regarding the ethylene receptor ETR2 (Fig. 2.3a), control fruits showed a peak at 7 DAT while treated fruits did not exhibit any peak and had a rising trend until 14 DAT according to a ripening delay. Finally, the expression trend of the ethylene response factor ERF2 (Fig. 2.3b) increased at ripening and was similar for both treatments; only a slight increase in ERF2
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

transcript levels was observed on days 7 and 11 in treated fruit. Previous work showed that ERF2 strongly respond to NAA and ethylene (Trainotti et al., 2007). This is in agreement with the possible increase in IAA levels (see below) in MJ-treated fruits.

Figure 2.3 Effect of Methyl-Jasmonate treatments on ethylene perception genes transcript levels. a, ETR2; b, ERF 2. ***, significant difference at P ≤ 0.001. Bars indicate mean ± standard error.

Present data show that MJ treatments reduce transcript abundance, though transiently, of ethylene biosynthetic (PpACO1 and PpACS1) and perception (PpETR2) genes which are strongly induced during ripening (Trainotti et al., 2006). The transient reduction of PpACO1, PpACS1 and PpETR2 transcript levels, may in part account for the reduction in softening and the delay in IAD progression in JA-treated fruit, as they remained at basal levels, until 7 DAT, typical of system 1 of ethylene biosynthesis (Barry et al., 2000). At both harvests, 11 and 14 DAT, a recovery in PpACO1, PpACS1 and PpETR2 transcript amount occurred, which reached and even overcame control levels which are compatible with system 2 of ethylene biosynthesis. A similar pattern was found by Ziosi et al. (2008a) in nectarines where MJ-treated fruits delayed the rise in climacteric ethylene production by a delay in the accumulation of PpACO1 transcript, thus delaying ripening. The observed rise in ACS1 transcripts can be due to an increase in auxin levels (see below), as recently demonstrated by Trainotti et al. (2007); auxin treatments in fact up-regulated ACS1 to a higher extent than ethylene. In the present study, IAD levels remained higher in MJ-treated fruits at both harvests suggesting that fruits were less ripe, probably because of a delayed initiation of climacteric ethylene production.
2.3.4 Exogenous MJ alters transcript levels of auxin biosynthesis and perception genes

Auxin-related gene expression was also differentially altered by exogenous MJ applications. In control fruit, transcript levels of tryptophan synthase β subunit (W synt), which is involved in the tryptophan-dependent IAA biosynthetic pathway, showed a peak at 7 DAT (Fig. 2.4a); in treated fruit, gene expression was mostly enhanced, in particular at ripening when it was more than double relative to controls. In controls, transcript amount of IGPS (indole-3-glycerol phosphate synthase), involved in the tryptophan independent IAA biosynthetic pathway (Fig. 2.4b) did not show substantial changes, and as for W synt transcript profile, MJ treatment enhanced gene expression especially at ripening. This pattern suggests a possible increase in IAA concentration in treated fruit.

Expression of GH3 (IAA-amino acid synthase), a member of a gene family that conjugates amino acids to IAA (Staswick et al. 2005 in Woodward and Bartel 2005) and likely serves to dampen the auxin signal by inactivating IAA via conjugation, showed increasing levels until ripening in control fruits (Fig. 2.5a). MJ only depleted transcript accumulation on day 7 suggesting a transient increase in IAA levels. Transcript levels of a gene responsible for IAA releasing from conjugates, IAA-amino acid amidohydrolase (Bartel and Fink 1995) showed a
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

peak on day 7 (Fig. 2.5b). In MJ-treated fruit gene expression was initially decreased but subsequently enhanced further suggesting an increase in IAA levels at ripening.

![Graph showing gene expression over days after treatment](image)

**Figure 2.5** Effect of MJ treatments on: a, IAA-amino acid synthase; b, and IAA-amino acid amidohydrolase. ***, significant difference at P ≤ 0.01; ****, P ≤ 0.001. Bars indicate mean ± standard error.

Messenger RNA levels of a putative TIR1 gene, coding for an auxin receptor (Dharmasiri et al. 2005), and of PIN1, a putative auxin efflux facilitator protein (Paponov et al. 2005) increased during the considered period until ripening (Fig. 2.6). In MJ-treated fruit, accumulation of both transcripts was inhibited on day 7; at ripening no significant differences were detected anymore.

![Graph showing gene expression over days after treatment](image)

**Figure 2.6** Effect of MJ treatments on: a, TIR1; b, PIN1. ***, significant difference at P ≤ 0.001. Bars indicate mean ± standard error.
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

IAA biosynthesis, metabolism and transport together ensure that appropriate auxin levels are in place to orchestrate plant development. Present data suggest that MJ induces a substantial change in auxin metabolism, possibly leading to increased biosynthesis, in RedHaven peaches, though its perception and transport appear only transiently affected. In ripening nectarine, the pattern of conjugate releasing and synthesizing genes suggest a possible increase in IAA levels in accord with the expression of IAA synthesizing genes (Trainotti et al., 2007). Significant increase in IAA content concomitant with climacteric ethylene production has been measured in RedHaven peaches (Miller et al., 1987). Trainotti et al. (2007) showed increasing IAA-related gene transcription during ripening along with climacteric ethylene production. In contrast, our data shows increased IAA biosynthesis and metabolism even though ripening is delayed as deduced by quality parameters and ethylene-related gene transcription (see above). Hence, this suggests that MJ might have a direct effect on IAA biosynthesis as deduced by the enhanced expression of W synt and IGPS 2 DAT concomitant with a repression of ethylene biosynthesis, whereas at 11 and 14 DAT the enhanced expression of ethylene and auxins occurs concomitant with a higher ethylene-related gene transcription.

2.3.5 Exogenous MJ alters the expression of other ripening-related genes

Other genes whose expression is altered during ripening were analyzed: allene oxide synthase (AOS), bZIP and 9-cis-epoxycarotenoid dioxygenase (NCED). In control fruit, the expression of bZIP transcription factor increased towards ripening (Fig. 2.7a). In MJ-treated fruits, an increasing trend in bZIP gene expression occurred, but delayed as compared with control fruits. NCED gene encodes for a key enzyme in the ABA biosynthetic pathway and its transcript levels correlate to ABA levels (Schwartz and Zeevaart, 2010). The potential contribution of ABA to the induction of fruit ripening was demonstrated in relation to ethylene in peach and grape (Zhang et al. 2009). PpNCED transcript levels increased at ripening in control fruit. MJ did not affect NCED expression (Fig. 2.7b) except at day 7 when it was totally depleted in treated fruit suggesting a decrease in ABA synthesis which is consistent with the lower ethylene production and the higher I\textsubscript{AD}.

AOS, a cytochrome P450 of the CYP74A family, is the first specific enzyme and the major control point of the JA biosynthetic pathway (Haga and Iino, 2004). In controls, AOS showed a
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

low level of expression and its transcript levels oscillated until ripening (Fig. 2.7c). MJ positively affected AOS expression especially on day 7 in line with the known positive feed-back regulation of the AOS enzyme (Kubisteltig et al., 1999) and this up-regulation is probably associated with an increase in JA production in treated fruit. A previous study showed that, in JA-treated nectarine in planta, an increase in AOS transcript levels occurred and was associated with an increase in endogenous jasmonic acid concentration 1 day after treatment (Ziosi et al., 2008a).

AOS gene expression is developmentally regulated (Kubigsteltig et al., 1999), and its message is up-regulated in response to wounding and treatments with JAs in leaves of Arabidopsis, tomato and tobacco (Laudert and Weiler, 1998; Howe et al., 2000) indicating that a positive feedback regulation in JA biosynthesis occurs leading to an amplification of the hormone signal (Laudert and Weiler, 1998).

**Figure 2.7** Effect of Methyl-Jasmonate treatments on: a, βZIP; b, NCED; c, AOS. ***, significant difference at P ≤ 0.01; ***, P ≤ 0.001. Bars indicate mean ± standard error.
In peaches, NCED genes are expressed only at the beginning of ripening when ABA accumulation is high, and precede the climacteric increase in ethylene production; once ABA starts to decrease, ethylene levels rise (Zhang et al., 2009a). MJ treatments strongly counteracted the rise in NCED gene expression, suggesting a slowing down of ripening that correlates with the reduced softening and delayed progression of $I_{AD}$ found in the present study. Trainotti et al. (2006) found in peaches that bZIP is a transcription factor up-regulated in the S3 to S4 transition stage. In MJ-treated fruits, bZIP remained at basal levels until harvest in accordance with the ripening delay effect found by quality trait assessments. Peach as well as other species accumulates JAs at ripening (Fan et al., 1998; Kondo et al., 2004; Ziosi et al., 2008a) and this is associated to higher transcription of $PpAOS1$. Ziosi et al. (2008a) showed a stimulation of AOS transcripts and an increase in JAs soon after the treatment and then both decreased during the considered time span; however, in this study, AOS transcript remained at a plateau in MJ-treated fruits while it transiently decreased in controls.

2.3.6 Pre-harvest MJ application negatively affects shelf-life

Ethylene production during shelf-life of treated and untreated fruits, divided in two groups of fruits by means of the DA-meter (non-climacteric: 0.9-1.2 $I_{AD}$; climacteric: 0.3-0.6 IAD), is presented in fig. 2.8. For both classes of fruits, MJ-treatments resulted in a higher ethylene production compared with controls throughout shelf-life. Ethylene production in treated non-climacteric fruits arrived to similar amounts than untreated climacteric fruits after 60 hours of shelf-life; while treated climacteric fruits produced more than 4-fold the ethylene produced by untreated climacteric fruits after the same time.
Pre-harvest methyl jasmonate application delays fruit ripening and alters ethylene and auxin biosynthesis and perception in peach

Figure 2.8 Effect of Methyl-Jasmonate treatments on shelf-life ethylene production of non-climacteric and climacteric fruits selected by the DA-meter. *, significant difference at P ≤ 0.05. Bars indicate mean ± standard error.

Shelf-life ethylene production data are in contrast with some reports suggesting that MJ treatments can extend fruit shelf-life (Peña-Cortéz et al., 2005) and protect them against pathogens and chilling injury (Yoshikawa et al., 2007) by delaying ripening and ethylene production, and up-regulating defense-related enzyme activities. In this study the differential accumulation of ethylene-related (mainly ACS and ETR2) and auxin biosynthetic gene transcript at harvest probably led to an increased ethylene production during shelf-life which negatively correlates with fruit shelf-life. This suggests that pre-harvest MJ treatments have to be regarded with caution in order to prevent undesired post-harvest effects.

In conclusion, JA-treated fruit confirms the down-regulation of crucial ripening-related genes, in agreement with the delayed progression of I_{AD}.
3. PRE- AND POST-HARVEST ABA APPLICATION INTERFERES WITH PEACH AND NECTARINE FRUIT RIPENING

3.1 Introduction

Abscisic acid (ABA), a well-known plant hormone, first discovered in the 1960’s under the names of either abscissin or dormin in young cotton fruits and in dormant buds of sycamore, plays key roles in seed and organ dormancy, plant responses to biotic and abiotic stress and sugar sensing (Schwartz and Zeevaart, 2010). ABA biosynthesis requires the cleavage of C\textsubscript{40} carotenoids to form its direct precursor (Nambara and Marion-Poll, 2005). This process, catalyzed by the 9-cis-epoxycarotenoid dehydrogenase enzyme (NCED), is the main rate-limiting step in ABA biosynthesis; in fact, alterations in NCED activity in deficient maize and over-expressing tomato mutants lead to reduced or enhanced ABA accumulation, respectively (Tan et al., 1997; Thompson et al., 2007).

ABA is also involved in developmental processes including fruit development and ripening (Zhang et al., 2009a and b). In fruits, ABA content is low during fruit development, increases towards ripening and decreases prior to harvest and senescence (Kondo and Gemma, 1993; Richardson and Cowan, 1995; Kondo and Kawai, 1998; Kondo et al., 2001a; Wang et al., 2007; Zhang et al., 2009a and b). In climacteric fruits, ABA accumulation precedes climacteric increase in ethylene production (Zhang et al. 2009a and b). Several studies demonstrated that exogenously applied ABA accelerates ripening of diverse species by enhancing color development (Kondo and Gemma, 1993; Jiang and Joyce, 2003; Cantín et al., 2007; Wang et al., 2007; Peppi et al., 2008), anthocyanin accumulation (Kondo and Gemma, 1993; Kondo and Kawai, 1998; Jeong et al., 2004; Wang et al., 2007; Peppi et al., 2008), flesh softening (Jiang and Joyce, 2003; Peppi et al., 2007), sugar accumulation (Kojima et al., 1995; Ofosu-Anim, 1996; Kondo and Kawai, 1998; Ofosu-Anim, 1998; Kobashi et al., 2001; Jeong et al., 2004) and chlorophyll degradation (Wang et al., 2005).

In the present work, peach was chosen as a model to shed some light on the physiological role of ABA during fruit ripening and shelf-life, and the reciprocal relationship between ethylene and ABA. The natural occurring ABA was applied to peach fruit at S3 and S3/S4 developmental stages under field conditions and in post-harvest to fruits differing on their maturity stage, and the
following were analysed: (i) ethylene production, fruit ripening and quality, (ii) postharvest behavior and (iii) stem water potential.

3.2 Materials and Methods

3.2.1 Plant material and experimental design

In field pre-harvest ABA treatments

Trials were conducted at the S. Anna experimental field of the University of Bologna and at a commercial orchard in Faenza, in Italy, and at the Kearney Agricultural Center of the University of California Davis, USA, on fifteen year old nectarine trees (Prunus persica L. Batsch), cv. Stark Red Gold, grafted on seedling rootstock, seven year old peach trees (Prunus persica L. Batsch) cv. Flaminia grafted on GF677 rootstock (Prunus persica x Prunus amigdalus), and eleven year old peach trees cv. O’Henry on nemaguard rootstock (Prunus persica x P. davidiana), respectively. In all sites, twenty trees were randomly selected for their size and fruit-load uniformity; in Italian sites, half of them were treated at mid-S3 growth stage and the remaining at S3/S4 transition stage; in the North American site 10 trees were treated at the end of S3 and at the S3/S4 transition stage. Treatments included 500 ppm ABA (Valent Biosciences) or water sprays (controls). Fruit growth and ripening evolution was followed after treatments. At harvest, the main fruit quality parameters were assessed. In ‘O’Henry’ trees only, mid-day water potential was determined periodically after treatments.

Post-harvest ABA fruit dipping

Two hundred ‘Stark Red Gold’ nectarines (Prunus persica L. Batsch) were harvested from fifteen year old nectarine trees grown at the S. Anna experimental station of the University of Bologna (Italy), and organized in different ripening classes (Table 3.1) as determined by means of the DA-meter. Half of the fruits in each class were dipped either in a 5 ppm ABA (Valent Biosciences) solution or water, left to ripen at controlled temperature (20ºC), and after 5 to 9 days of shelf-life, the main quality parameters were evaluated. Also, ethylene production was followed in fruits belonging to two different ripening classes (1.3-0.9 and 0.8-0.5 I<sub>AD</sub>) after the treatment.
Table 3.1 ABA dipping treatments in fruits at different developmental stages belonging to different I<sub>AD</sub> classes

<table>
<thead>
<tr>
<th>Ripening Stage</th>
<th>I&lt;sub&gt;AD&lt;/sub&gt;-class</th>
<th>Treatment</th>
<th>Shelf-life duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-climacteric</td>
<td>1.6 – 1.4</td>
<td>5 ppm ABA</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Non-climacteric</td>
<td>1.3 – 0.9</td>
<td>5 ppm ABA</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Non-climacteric</td>
<td>1.2 – 1.0</td>
<td>5 ppm ABA</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Onset of climacteric</td>
<td>1.0 – 0.8</td>
<td>5 ppm ABA</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>Climacteric</td>
<td>0.8 – 0.5</td>
<td>5 ppm ABA</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

3.2.2 Quality trait, ethylene and I<sub>AD</sub> determination

The main fruit quality traits, flesh firmness (FF), soluble solids content (SSC) and titratable acidity (TA), as well as I<sub>AD</sub> and ethylene were determined as previously described in point 2.2.2, with slight differences in the brands or models of the equipment used to assess SSC, FF and ethylene. Also, in the North American trial skin color, as determined by L*, C* and H° color space, was assessed using a Minolta CR-300 colorimeter (Minolta, Osaka, Japan) by measuring the two opposite cheeks of each fruit. L* is the lightness and corresponds to a black-white scale (0, black; 100. White), H° is the hue angle on the color wheel, and C* is the chroma, a measure of color intensity, which begins with zero (achromatic) and increases with intensity (McGuire, 1992).

3.2.3 Tree water status evaluation

Midday stem water potential (Ψ) of ABA-treated and untreated 'O’Henry’ trees was measured using a Scholander pressure chamber (Soilmoisure Equipment Corp., Santa Barbara, CA, USA), on 3 basal leaves per tree, twice a week, following the method described by McCutchan and Shackel (1992). Prior to excision and measurement, leaves were enclosed in aluminium foil-covered plastic bags on the tree for at least one hour to allow equilibration with the stem.
3.2.4 Statistical analysis

All data were statistically analyzed using a completely randomized design. For field ‘Stark Red Gold’ and ‘O’Henry’ trials 2 factors were considered: the treatment (2 levels: ABA and control) and the treatments date (2 levels: S3 and S3/S4 stages); while for ‘Flaminia’ only the treatment was considered as a factor (3 levels: ABA in S3, ABA in S3/S4 and control). For post-harvest dipping the treatment (2 levels: ABA and control) and ripening class (2 levels: 0.5 - 0.8 and 0.9 - 1.3 for 5 days of shelf-life; and 3 levels: 0.8 – 1.0, 1.0 - 1.2 and 1.4 – 1.6 for 9 days of shelf-life) were considered. When significant interaction occurred, the treatment factor was analyzed separately per each level of treatment date or ripening class, for pre-harvest and post-harvest treatments, respectively. Mean separation analysis was performed by the Student Newman-Keuls test. Pearson correlation analyses were performed to evaluate the relation between main quality parameters with DA-meter analysis.

3.3 Results and Discussion

3.3.1 Field ABA application increases fruit size and skin color intensity

The effects of pre-harvest ABA treatment on peach and nectarine fruit quality traits at harvest are presented in Tables 3.2 to 3.6. In ‘Flaminia’ peaches and ‘Stark Red Gold’ nectarines, mid-S3 treatments significantly increased fruit weight without altering FF, SSC or TA as compared with controls while S3/S4 treatments had no effects on any trait. In O’Henry peaches, ABA-treated fruits in both stages exhibited higher color intensity as determined by lower values of L, C and H° at both treatment dates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm⁻²)</th>
<th>SSC (ºBrix)</th>
<th>TA (g l⁻¹)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.56 a</td>
<td>9.06 a</td>
<td>6.13 a</td>
<td>235 b</td>
</tr>
<tr>
<td>S3-treated</td>
<td>4.75 a</td>
<td>9.40 a</td>
<td>6.86 a</td>
<td>266 a</td>
</tr>
<tr>
<td>S3/S4-treated</td>
<td>4.92 a</td>
<td>9.06 a</td>
<td>7.13 a</td>
<td>255 ab</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at P ≤ 0.05. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
Table 3.3 Effect of ABA treatments carried out in mid-S3 on ‘Stark Red Gold’ nectarine quality traits at harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm(^{-2}))</th>
<th>SSC (ºBrix)</th>
<th>Size (mm)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.05 a</td>
<td>14.0 a</td>
<td>67.0 a</td>
<td>177 b</td>
</tr>
<tr>
<td>Treated</td>
<td>5.04 a</td>
<td>13.9 a</td>
<td>68.6 a</td>
<td>196 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at P ≤ 0.05. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 3.4 Effect of ABA treatments carried out in S3/S4 transition on ‘Stark Red Gold’ nectarine quality traits at harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm(^{-2}))</th>
<th>SSC (ºBrix)</th>
<th>Size (mm)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.37 a</td>
<td>13.0 a</td>
<td>68.1 a</td>
<td>183 a</td>
</tr>
<tr>
<td>Treated</td>
<td>6.02 a</td>
<td>13.2 a</td>
<td>67.2 a</td>
<td>176 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 3.5 Effect of ABA treatments carried out in late S3 on ‘O’Henry’ peach quality traits at harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I(_{AD})</th>
<th>FF (kg cm(^{-2}))</th>
<th>Weight (g)</th>
<th>SSC (ºBrix)</th>
<th>L</th>
<th>C</th>
<th>H°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00 a</td>
<td>5.95 a</td>
<td>242.0 a</td>
<td>10.7 a</td>
<td>56.6 a</td>
<td>39.8 a</td>
<td>60.5 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.10 a</td>
<td>5.90 a</td>
<td>236.4 a</td>
<td>10.3 a</td>
<td>53.2 b</td>
<td>37.9 b</td>
<td>54.0 b</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

n.s., not significant; **, significant difference at P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 3.6 Effect of Abscisic acid treatments carried out in S3/S4 on ‘O’Henry’ peach quality traits at harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I(_{AD})</th>
<th>FF (kg cm(^{-2}))</th>
<th>Weight (g)</th>
<th>SSC (ºBrix)</th>
<th>L</th>
<th>C</th>
<th>H°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.18 a</td>
<td>6.04 a</td>
<td>238.8 a</td>
<td>11.2 a</td>
<td>58.5 a</td>
<td>40.4 a</td>
<td>67.5 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.13 a</td>
<td>5.72 a</td>
<td>247.7a</td>
<td>11.2 a</td>
<td>53.8 b</td>
<td>38.1 b</td>
<td>57.8 b</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

n.s., not significant; **, significant difference at P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
Pre- and post-harvest ABA application interferes with peach and nectarine fruit ripening

Relations between traditional quality parameters and I_{AD} in ‘O’Henry’ peaches are presented in figure 3.1. The index correlated negatively with ethylene production ($r = -0.701$) and positively with FF and flesh hue angle ($H^\circ; r=0.874$ and $0.950$, respectively). No correlations were found with SSC, or other color parameters (skin L*C*H* and flesh L*C*). This results suggest that fruits with low I_{AD} present lower firmness and flesh $H^\circ$ and produce more ethylene than fruits with higher I_{AD}, suggesting that they are riper. This data, together with those of Ziosi et al. (2008b), further support the validity of the I_{AD} as a reliable maturity index for peach and nectarine fruits.

![Graphs showing correlations between I_{AD} measurements with ethylene production (a), FF (b) and flesh H° in O’Henry peaches.](image)

Figure 3.1 Correlation between I_{AD} measurements with ethylene production (a), FF (b) and flesh H° in O’Henry peaches. $r$, Pearson correlation coefficient. ***, linear correlation significant at $P \leq 0.001$.**

Thus, under present experimental conditions, pre-harvest ABA application mainly influences fruit weight and skin color. Present data are in accord with previous literature which reports that exogenous ABA affected fruit growth in peach (Kobashi et al., 1999) and color development in litchi (Jiang and Joyce, 2003), sweet cherry (Kondo and Gemma, 1993) and grapes (Peppi et al., 2008). In peaches, fruit growth is sustained by about 30% by phloem and 70 % by xylem inflows.
while transpiration accounts for 55-65 % of total inflows (Morandi et al., 2007a). Transpiration enhances carbohydrate and water imports, thus enhancing fruit growth (Morandi et al., 2010a), as it generates a pressure gradient that favors passive phloem unloading and water intake (Morandi et al., 2007). The fact that ABA-treated fruit are larger than controls but have the same SSC content suggests that ABA enhances fruit enlargement by inducing both water and carbohydrates uptake. In fact, one of the documented effects of ABA concerns phloem carbohydrate unloading (Ofosu-anim et al., 1996; Ofosu-anim et al., 1998; Kobashi et al., 2001; Peng et al., 2003; Pan et al., 2005; Pan et al., 2006). Regarding color improvement, previous studies with exogenous ABA treatments showed skin color enhancement in grapes (Peppi et al., 2007; Peppi et al., 2008), litchi (Wang et al., 2005; Wang et al., 2007), strawberries (Jiand and Joyce, 2003) and sweet cherries (Kondo and Gemma, 1993) due to anthocyanin accumulation and/or chlorophyll degradation. However, under present conditions, ABA treatments induced leaf abscission and, thus, increased light penetration into the canopy. In our study, the increased light penetration could account for the improved fruit color since the development of anthocyanins, the pigment responsible for red color development in the peach skin, is greatly influenced by solar radiation (Loreti et al., 1993).

3.3.2 Post-harvest ABA treatments alter ripening-related parameters

The effect of post-harvest ABA treatments on shelf-life ethylene production is presented in figure 3.2. In climacteric fruits (IA between 0.5-0.8), ethylene production was greatly inhibited by ABA dipping after 2 and 5 days of shelf-life, and treated fruits produced ~20 % of the ethylene produced by controls. In non-climacteric fruits (0.9-1.3), there were no significant differences in ethylene emission between treatments though ethylene production tended to be lower in ABA-treated fruits at 1 and 2 days of shelf-life.
Pre- and post-harvest ABA application interferes with peach and nectarine fruit ripening

Figure 3.2 Ethylene production during shelf-life after post-harvest ABA dipping. a, 0.5-0.8 I<sub>AD</sub>; b, 0.9-1.3 I<sub>AD</sub>. *, significant difference at P ≤ 0.05; **, P ≤ 0.01. Bars indicate mean ± standard error.

As far as quality traits are concerned, two situations were found (Tables 3.7 and 3.8). After 5 days of shelf-life, no significant differences in FF, SSC and TA were recorded between treatments while FF and TA were significantly different between climacteric (0.5-0.8 I<sub>AD</sub>) and non-climacteric fruits (0.9-1.3 I<sub>AD</sub>). After a longer shelf-life period (9 days), ABA-treated fruits exhibited higher SSC and similar values of FF and TA in all ripening classes as compared to controls while FF and TA were significantly different between ripening classes with less ripen fruits (I<sub>AD</sub> between 1.4-1.6) presenting the highest TA and FF, the intermediate class (I<sub>AD</sub> between 1.0-1.2) exhibiting intermediate TA, and similar values of FF than the ripest fruits (I<sub>AD</sub> between 0.8-1.0); the latter one also had the lowest TA.

Table 3.7 Effect of post-harvest ABA treatments on fruit quality traits after 5 days of shelf-life.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm&lt;sup&gt;-3&lt;/sup&gt;)</th>
<th>SSC (ºBrix)</th>
<th>TA (g l&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.65 a</td>
<td>13.1 a</td>
<td>11.7 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.73 a</td>
<td>12.9 a</td>
<td>11.6 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I&lt;sub&gt;AD&lt;/sub&gt; Class</th>
<th>FF (kg cm&lt;sup&gt;-3&lt;/sup&gt;)</th>
<th>SSC (ºBrix)</th>
<th>TA (g l&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 - 0.8</td>
<td>1.08 b</td>
<td>13.1 a</td>
<td>11.0 b</td>
</tr>
<tr>
<td>0.9 - 1.3</td>
<td>2.28 a</td>
<td>12.9 a</td>
<td>12.2 a</td>
</tr>
<tr>
<td>Significance</td>
<td>***</td>
<td>n.s.</td>
<td>*</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at P ≤ 0.05; ***, P ≤ 0.001. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
Pre- and post-harvest ABA application interferes with peach and nectarine fruit ripening

Table 3.8 Effect of post-harvest ABA treatments on fruit quality traits after 9 days of shelf-life.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>TA (g l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.90 a</td>
<td>12.8 b</td>
<td>10.6 a</td>
</tr>
<tr>
<td>Treated</td>
<td>0.99 a</td>
<td>14.0 a</td>
<td>10.3 a</td>
</tr>
</tbody>
</table>

**Significance**

<table>
<thead>
<tr>
<th>I(_{AD}) Class</th>
<th>n.s.</th>
<th>**</th>
<th>n.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 – 1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 – 1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4 – 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.s., not significant; **, significant at P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Recent reports indicate that post-harvest ABA treatments induce ethylene production and enhance ripening of peaches by triggering ethylene production whereas fluridone, an inhibitor of ABA synthesis, inhibits ethylene production and delays ripening (Zhang et al., 2009b). In contrast, our data shows that ethylene production is mainly inhibited by ABA treatments especially in climacteric fruits (0.5 < I\(_{AD}\) < 0.8) whereas SSC is enhanced by the treatment after 9 days of shelf-life. This opposite effect could be due to the ripening stage of the fruits used, which, in our experiment, were at the onset of ethylene production or already producing ethylene; thus ABA seems to interfere with ethylene production once its biosynthetic machinery is active. In apples, Kondo et al. (2001b) found that post-harvest ABA treatments enhance ethylene production in pre-climacteric, climacteric and post-climacteric fruits, with a greater effect in the more immature fruits. Borsani et al., (2009) found that in ABA-treated peaches SSC remained unaffected after harvest. In our study, according to the literature, the main effect of ABA concerned SSC which during shelf-life remained higher in treated fruits as compared with control ones.

3.3.3 Field ABA applications modify stem water potential of peach trees

Mid-day stem Ψ is presented in figure 3.3. Both control and treated trees showed a similar pattern after treatments; in the late S3, stem Ψ decreased from 1 to 11 DAT as a consequence of soil water depletion due to tree transpiration, then it increased due to irrigation, and finally it
decreased again until 17 DAT. In the S3/S4, there were no major changes in stem Ψ between measurement dates. ABA-treated trees showed higher levels of stem Ψ throughout the experimental period.

Figure 3.3 Effect of pre-harvest ABA treatments done at late-S3 (a) and S3/S4 transition (b) stages on stem Ψ. **, significant difference at P ≤ 0.01; ***, P ≤ 0.001.

Present data, shows that pre-harvest ABA application influences peach tree stem Ψ. Water potential measures the tendency of the water to move through the soil-plant-air continuum, thus provide a sensitive indicator of daily and seasonal changes in plant water status (Blake et al., 1996; Williams and Araujo, 2002; Naor and Cohen, 2003). Choné et al. (2001) indicated that stem Ψ is the first indicator of water stress in field-grown grapevines. In the latter, water potential correlated with irrigation treatments, and deficit irrigated plants had lower Ψ values as compared with well irrigated plants (Williams and Araujo, 2002). Thus, the higher stem Ψ exhibited in treated trees suggests that they have transpired less water than control plants. These results correlate with the well-known role of ABA in stomatal closure as demonstrated in ABA-deficient mutants and by exogenous ABA treatments in diverse species (Iuchi et al., 2001; Qin and Zeevaart, 2002; Li et al., 2004; Thompson et al., 2007; Ma et al., 2008). The differences in tree transpiration rates became evident soon after the treatment; in fact, differences in stem Ψ were apparent from 1 DAT. In other species, exogenous ABA triggers fast stomata responses that lead to closure within 8-20 minutes in Vicia faba (Roelfsema et al., 2004)
In conclusion, both pre-harvest and post-harvest ABA treatments lead to larger, sweeter and more colored fruits. This is supported by the finding that ABA-treated peach trees retain more water. New perspectives are open for the use of ABA under field conditions.
ABA and PDJ treatments during fruit development improve Kiwifruit quality

4. ABA AND PDJ TREATMENTS DURING FRUIT DEVELOPMENT IMPROVE KIWIFRUIT QUALITY

4.1 Introduction

The kiwifruit berry accumulates large amounts of carbon as starch during fruit development (Walton and De Jong, 1990). Its quality has been classically defined in terms of dry matter accumulation as it correlates with starch content (Jordan et al., 2000) and ripe fruit soluble solids (Burdon et al., 2004). However, the quality of the recent kiwifruit cvs Hort16A and Jintao, of the species Actinidia chinensis, is defined in terms of flesh color progression as they are characterized by the development of a bright yellow flesh during ripening (Montefiori et al., 2009).

During kiwifruit development three main stages of sugar metabolism occur: cell division, starch accumulation and fruit maturation (Richardson et al., 2004). In the first stage, most of the carbon is allocated to structural components, fruit RGR is at its maximum and sucrose synthase (SuSy) activity prevails over invertases. In the second period, a large portion of carbon is allocated as starch, which abruptly rises at about 45 DAFB, following the peak of glucose, and rises rapidly towards 120 DAFB. The final period, that starts 120 DAFB with the stop of starch accumulation, marks the onset of ripening which is followed by rapid starch degradation (Moscatello et al., 2011). Soluble sugar concentrations peak during cell division, declines during starch accumulation and increases again towards maturation, when accumulated starch is hydrolyzed into sugars (Nardozza et al., 2010).

Within the genus Actinidia a range of fruit color occurs, including green, red, purple, yellow and orange (McGhie and Ainge, 2002). This difference in color between fruits of A. deliciosa and A. chinensis is due only to the degradation of flesh chlorophyll in the latter one during ripening, as there are no differences in carotenoid composition (McGhie and Ainge, 2002; Nishiyama et al., 2008; Montefiori et al., 2009).

Jasmonates (JAs) and abscisic acid (ABA) are plant growth regulators that mediate plant responses to stress and are involved in fruit development and ripening (Wasternack, 2007; Zhang et al., 2009a; Schwartz and Zeevaart, 2010). Exogenous application of either one has demonstrated to alter color development, anthocyanin synthesis, chlorophyll degradation, soluble solid accumulation and softening in several species (Kondo and Gemma, 1993; Fan et al., 1998b;
ABA and PDJ treatments during fruit development improve Kiwifruit quality

Kondo et al., 2001b; Kondo et al., 2002; Jiang and Joyce, 2003; Kondo et al., 2005a; Wang et al., 2005; Wang et al., 2007; Peppi et al., 2008; Ziosi et al., 2008a).

As kiwifruits are late season fruits, fruit growers pick them as early as possible to avoid frost and bad weather conditions (Beever and Hopkirk, 1990). However, this practice can incur in harvesting immature fruits with poor color, flavor and shelf life that will never reach an excellent eating quality (Tromp, 2005). For this reason, exogenous ABA and JAs were used in both Actinidia deliciosa and Actinidia chinensis to further shed light on their role in the control of ripening and harvest timing.

4.2 Materials and Methods

4.2.1 Plant material and experimental design

Actinidia deliciosa: pre-harvest ABA treatments

The trial was carried out during two consecutive seasons (2009 and 2010) at the S. Anna experimental field of the University of Bologna, Italy, on 7-years old kiwifruit (Actinidia deliciosa [A. Chev.] C.F. Liang et A.R. Ferguson var. deliciosa) cv. ‘Hayward’ vines trained under a T-bar system. In the first season, two types of experiments were carried out by spraying either water or a 500 ppm ABA (S-(+)-abscisic acid; Valent Biosciences, Libertyville, IL, USA) solution to 5 randomly selected vines or to 20 girdled branches randomly selected from 5 vines. In the second season, 24 girdled branches randomly selected from 4 vines were sprayed with 500 ppm ABA or water at different stages of fruit development (Table 4.1).
**ABA and PDJ treatments during fruit development improve Kiwifruit quality**

Table 4.1 Scheme of *Actinidia delicosa* treatments.

<table>
<thead>
<tr>
<th>Trial Season</th>
<th>Plant Material</th>
<th>Treatment date</th>
<th>Treated unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 -2009</td>
<td>Full vine</td>
<td>4 MAFB</td>
<td>5 vines</td>
</tr>
<tr>
<td></td>
<td>Girdled branches</td>
<td>4 MAFB</td>
<td>20 branches (5 vines)</td>
</tr>
<tr>
<td>2009 – 2010</td>
<td>Girdled branches</td>
<td>1 MAFB</td>
<td>24 branches (4 vines)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 MAFB</td>
<td>24 branches (4 vines)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 MAFB</td>
<td>24 branches (4 vines)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 MAFB</td>
<td>24 branches (4 vines)</td>
</tr>
</tbody>
</table>

1 MAFB, months after full bloom.

The girdling procedure was performed by removing with a knife a 5-mm section of phloem of well exposed branches that had at least 4 fruits the day before the treatments; afterwards, branches were arranged to obtain a fruit-to-leaf ratio of 2 by pruning and removing exceeding leaves. Treated fruit were harvested about 5 months after full bloom (MAFB), after achieving the industry requirements, and main quality parameters were evaluated; also, a fruit sample (60 fruits per treatment) was stored at 1°C for 2 months and quality traits were assessed. In addition, in full vines, gas exchange parameters and water use efficiency were evaluated in 2009, the treatment day, and 1 and 2 days after the treatment and, in girdled branches, the treatment day and the day after.

**Actinidia chinensis: pre-harvest ABA and PDJ treatments**

Trials were carried out during two consecutive seasons (2009-2010) at the S. Anna experimental field of the University of Bologna, Italy, on 2 to 3-year old gold kiwifruit (*Actinidia chinensis* Planch. var. chinensis) cv. ‘Jintao’® vines trained under a GDC system. In both seasons, 15 plants were randomly selected to be sprayed with 500 ppm ABA (Valent Biosciences), 200 ppm PDJ (Fine Agrochemicals Limited, Worcester, UK), or water (controls), at the beginning of yellow flesh color development in gold kiwifruits (~ 2 weeks before harvest).

Fruits were harvested following industry requirements in both years, and main fruit quality parameters were assessed. Moreover, in the first year, a delayed harvest was carried out 1 week after the commercial one. Finally, in both seasons, a fruit sample (60 fruits per treatment) was stored for two months at 1°C to evaluate further changes in fruit quality traits.
Actinidia chinensis: post-harvest ABA and PDJ dipping

Three hundred ‘Jintao’® gold kiwifruits were harvested from two-years old kiwifruit vines grown at the S. Anna experimental station of the University of Bologna (Italy) and distributed in the different treatments (50 fruits each) that consisted in 1-min fruit dipping in the following solutions: 20 ppm ABA (Valent Biosciences), 200 ppm ABA, 20 ppm PDJ (Fine Agrochemicals Limited), 200 ppm PDJ, 20 ppm ABA plus 20 ppm PDJ, or in water. Afterwards fruits were taken to a storage cell and stored at 1°C. Fruit quality traits were evaluated on 20 fruits per treatment after two months of storage.

4.2.2 Quality traits and I_{AD} determinations

The main fruit quality traits such as flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA), flesh color (Hue angle, H°) and dry matter (DM) content were determined. FF, SSC and TA were measured as previously described in point 2.2.2. Whereas, H° was determined using a Minolta colorimeter CR 200 (Minolta Corp., Osaka, Japan) on two faces per each fruit, after removing a 2 mm-thick skin layer. DM was assessed on 10 g center-transversal slice by oven-drying until the fruit weight remain constant (approximately for 3 days) at the temperature of 65°C, in accordance with the kiwistart protocol (Montefiori, 2003).

Additionally, the extent of fruit ripening was non-destructively measured by means of the Kiwi-meter, a modified DA-meter (see point 2.2.2) specially realized for kiwifruits and differing from the DA-meter only by the wavelengths used. Both the Kiwi-meter and the index were developed and patented by the Fruit Tree and Woody Plant Sciences Department of the University of Bologna (Costa et al., 2009). The used wavelength differs between A. chinensis and A. deliciosa fruits, and is calculated as follows:

\[ I_{AD} = A_1 - A_2, \]

where A1 and A2 are the absorbance values at the wavelengths of 640 and 800 for yellow kiwifruits and 540 and 800 for the green ones. In A. deliciosa, the I_{AD} ranges from 0.2 (unripe fruits) up to 2.0 (fully ripe fruits), whereas in A. chinensis there is a strict negative relation between external and flesh I_{AD} with flesh H° (Costa et al., 2010).
4.2.3 Gas exchange measurement

Gas exchange characteristics as net photosynthesis (A), transpiration (E) and stomatal conductance ($g_s$) were measured with a LI-COR 6400 portable photosynthesis system (Li-Cor Inc. Lincoln, Neb, USA). Three well exposed and fully expanded leaves per vine, in full vine treatments, and 1 leaf per girdled branch were selected and measured. Measurements were made on sunny days, between 11:00 a.m. and 1:00 p.m. using a Q-beam (blue and red diode) light source set as 1000 $\mu$mol m$^{-2}$ s$^{-1}$ which was found to be saturating for A. deliciosa under our field conditions. Also, instantaneous water use efficiency (WUEi = $A/E$) was assessed.

4.2.4 Statistical analysis

All data were statistically analyzed using a completely randomized design. In ‘Hayward’ 2009 trials, the factor was the treatment (2 levels: ABA and control) while for 2010 trials, the factors were the treatment (2 levels: ABA and control) and the time after treatment (4 levels: 1, 2, 3 and 4 month after treatment). For ‘Jintao’ experiments, the factor was the treatment (3 levels: ABA, PDJ and control for pre-harvest treatments; 6 levels: ABA 20 ppm, ABA 200 ppm, PDJ 20 ppm, PDJ 200 ppm, ABA 20 ppm + PDJ 20 ppm and control for post-harvest treatment). When significant interactions occurred, the treatment factor was analyzed separately per each level of treatment application time. Mean separation analysis was performed by the Student Newman-Keuls test. Pearson correlation analysis was performed to evaluate the relation between main quality parameters with Kiwi-meter analysis.

4.3 Results and Discussion

4.3.1 Field ABA application improves quality and size of Actinidia deliciosa fruits

Girdled branch treatments (Table 4.2 to 4.6) showed that only late applications (4 MAFB), had positive effects on fruit quality traits; in both seasons this treatment enhanced fruit weight, and in 2010 also significantly enhanced I$_{AD}$, size and softening. When applied earlier (1 MAFB), ABA increased I$_{AD}$ and FF, 2 MAFB decreased size, weight, SSC, I$_{AD}$ and DM while no significant effects were observed when applied 3 MAFB. When applied to full vines, late season treatments had no effects on fruit weight or quality traits (Table 4.7).
**Table 4.2** Effects of 2010 ABA treatments applied 1 MAFB to ‘Hayward’ girdled branches on main quality traits at harvest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size (mm)</th>
<th>Weight (g)</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>TA (g l(^{-1}))</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.1 a</td>
<td>130 a</td>
<td>1.20 b</td>
<td>8.10 b</td>
<td>6.47 a</td>
<td>22.7 a</td>
<td>17.7 a</td>
</tr>
<tr>
<td>Treated</td>
<td>78.1 a</td>
<td>133 a</td>
<td>1.26 a</td>
<td>8.86 a</td>
<td>6.73 a</td>
<td>24.3 a</td>
<td>17.9 a</td>
</tr>
</tbody>
</table>

*Significance* n.s., not significant; *, significant difference at \( P \leq 0.05 \); ***, \( P \leq 0.001 \). Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).

**Table 4.3** Effects of 2010 ABA treatments applied 2 MAFB to ‘Hayward’ girdled branches on main quality traits at harvest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size (mm)</th>
<th>Weight (g)</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>TA (g l(^{-1}))</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.1 a</td>
<td>129 a</td>
<td>1.23 a</td>
<td>8.60 a</td>
<td>6.56 a</td>
<td>23.3 a</td>
<td>17.5 a</td>
</tr>
<tr>
<td>Treated</td>
<td>73.5 b</td>
<td>115 b</td>
<td>1.18 b</td>
<td>8.34 a</td>
<td>5.92 b</td>
<td>22.8 a</td>
<td>15.7 b</td>
</tr>
</tbody>
</table>

*Significance* * *, significant difference at \( P \leq 0.05 \); **, \( P \leq 0.01 \). Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).

**Table 4.4** Effects of 2010 ABA treatments applied 3 MAFB to ‘Hayward’ girdled branches on main quality traits at harvest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size (mm)</th>
<th>Weight (g)</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>TA (g l(^{-1}))</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.0 a</td>
<td>122 a</td>
<td>1.15 a</td>
<td>8.70 a</td>
<td>6.56 a</td>
<td>22.4 a</td>
<td>17.1 a</td>
</tr>
<tr>
<td>Treated</td>
<td>75.9 a</td>
<td>129 a</td>
<td>1.16 a</td>
<td>8.46 a</td>
<td>6.24 a</td>
<td>22.9 a</td>
<td>16.4 a</td>
</tr>
</tbody>
</table>

*Significance* n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).

**Table 4.5** Effect of 2009 ABA treatments applied 4 MAFB to ‘Hayward’ girdled branches on main fruit quality traits at harvest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight (g)</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>TA (g l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>109 b</td>
<td>6.90 a</td>
<td>11.5 a</td>
<td>23.0 a</td>
</tr>
<tr>
<td>Treated</td>
<td>117 a</td>
<td>6.83 a</td>
<td>12.0 a</td>
<td>23.9 a</td>
</tr>
</tbody>
</table>

*Significance* *, significant difference at \( P \leq 0.05 \). Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).
ABA and PDJ treatments during fruit development improve Kiwifruit quality

Table 4.6 Effects of 2010 ABA treatments applied 4 MAFB to ‘Hayward’ girdled branches on main quality traits at harvest

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size (mm)</th>
<th>Weight (g)</th>
<th>IAD</th>
<th>FF (kg cm⁻³)</th>
<th>SSC (°Brix)</th>
<th>TA (g l⁻¹)</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.9 b</td>
<td>129 a</td>
<td>1.15 a</td>
<td>8.24 a</td>
<td>7.40 a</td>
<td>24.3 a</td>
<td>17.2 a</td>
</tr>
<tr>
<td>Treated</td>
<td>78.5 a</td>
<td>139 b</td>
<td>1.22 b</td>
<td>7.66 b</td>
<td>8.65 b</td>
<td>22.0 a</td>
<td>17.1 a</td>
</tr>
</tbody>
</table>

Significance: ***, P ≤ 0.001; **, P ≤ 0.01; *, P ≤ 0.05; n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

After 2 months of cold storage, ABA treatments affected kiwifruit ripening (table 4.8 to 4.13). One MAFB-treated fruits showed a delayed softening as compared to control ones; 2 and 3 MAFB-treated fruits exhibited reduced SSC and DM content, as well as 4 MAFB full vine treated fruits as compared to control ones; the latter treatment, when applied to girdled branches, showed no significant differences or a reduced SSC as compared to control fruits, the first and second season, respectively. Full vine-treated fruits also showed a reduced I

Table 4.7 Effect of 2009 ABA treatments to ‘Hayward’ vines on main fruit quality traits and yield at harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight (g)</th>
<th>FF (kg cm⁻³)</th>
<th>SSC (°Brix)</th>
<th>TA (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102 a</td>
<td>6.70 a</td>
<td>11.1 a</td>
<td>24.6 a</td>
</tr>
<tr>
<td>Treated</td>
<td>103 a</td>
<td>6.56 a</td>
<td>11.1 a</td>
<td>25.2 a</td>
</tr>
</tbody>
</table>

Significance: n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 4.8 Effects of 2010 ABA treatments done 1 MAFB to ‘Hayward’ girdled branches on main quality traits after cold storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IAD</th>
<th>FF (kg cm⁻³)</th>
<th>SSC (°Brix)</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.26 a</td>
<td>2.91 b</td>
<td>13.0 a</td>
<td>19.1 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.28 a</td>
<td>3.75 a</td>
<td>12.8 a</td>
<td>18.8 a</td>
</tr>
</tbody>
</table>

Significance: n.s., not significant; *, significant difference at P ≤ 0.05. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
AB and PDJ treatments during fruit development improve Kiwifruit quality

Table 4.9 Effects of 2010 ABA treatments done 2 MAFB to ‘Hayward’ girdled branches on main quality traits after cold storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.27 a</td>
<td>3.34 a</td>
<td>13.0 b</td>
<td>19.4 b</td>
</tr>
<tr>
<td>Treated</td>
<td>1.30 a</td>
<td>3.67 a</td>
<td>11.7 a</td>
<td>17.9 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>***</td>
<td>**</td>
</tr>
</tbody>
</table>

n.s., not significant; ***, significant difference at \( P \leq 0.001 \). Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).

Table 4.10 Effects of 2010 ABA treatments done 3 MAFB to ‘Hayward’ girdled branches on main quality traits after cold storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.27 a</td>
<td>3.05 a</td>
<td>12.4 a</td>
<td>18.2 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.31 a</td>
<td>2.99 a</td>
<td>11.1 b</td>
<td>16.7 b</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at \( P \leq 0.05 \); **, \( P \leq 0.01 \). Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).

Table 4.11 Effects of 2009 ABA done 4 MAFB treatments to ‘Hayward’ girdled branches on main quality traits after cold storage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>TA (g l(^{-1}))</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.22 a</td>
<td>2.99 a</td>
<td>14.3 a</td>
<td>12.4 a</td>
<td>18.4 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.24 a</td>
<td>2.74 a</td>
<td>14.1 a</td>
<td>12.3 a</td>
<td>17.6 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).

Table 4.12 Effects of 2010 ABA treatments done 4 MAFB to ‘Hayward’ girdled branches on main quality traits after 2 months of cold storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( I_{AD} )</th>
<th>FF (kg cm(^{-3}))</th>
<th>SSC (°Brix)</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.26 a</td>
<td>2.58 a</td>
<td>13.3 a</td>
<td>18.7 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.24 a</td>
<td>2.47 a</td>
<td>12.7 b</td>
<td>18.1 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at \( P \leq 0.05 \). Data represent mean values. In each column, means followed by the same letter are not statistically different (at \( P \leq 0.05 \)).
ABA and PDJ treatments during fruit development improve Kiwifruit quality

Table 4.13 Effects of ABA treatments to ‘Hayward’ vines on main quality traits after cold storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IAD</th>
<th>FF (kg cm$^{-3}$)</th>
<th>SSC (ºBrix)</th>
<th>TA (g l$^{-1}$)</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.21 b</td>
<td>2.54 a</td>
<td>14.3 a</td>
<td>16.2 a</td>
<td>18.0 a</td>
</tr>
<tr>
<td>Treated</td>
<td>1.10 a</td>
<td>2.49 a</td>
<td>13.8 a</td>
<td>14.2 a</td>
<td>16.8 b</td>
</tr>
</tbody>
</table>

Significance

***, significant difference at $P \leq 0.001$ Data represent mean values. In each column, means followed by the same letter are not statistically different (at $P \leq 0.05$).

n.s., not significant

In the present study, exogenous ABA treatments alter fruit weight, sugar content and dry matter accumulation, according to differences in fruit physiology and growth pattern. Upon treatment date, ABA can increase (4 MAFB), decrease (2 MAFB) or not affect (1 MAFB and 3 MAFB) fruit weight, sugar and dry matter content at harvest. In fact, several reports indicate that exogenous ABA treatments increase sugar content in many species by enhancing assimilate uptake and sugar metabolism (Ofosu-Anim et al., 1994; Ofosu-Anim et al., 1996; Ofosu-Anim et al., 1998; Kobashi et al., 1999; Kobashi et al., 2001). Moscatello et al. (2005) indicated that accelerating the onset of starch accumulation and starch degradation can anticipate soluble solids accumulation and harvest.

Present data show that exogenous ABA enhances ripening when fruit sugar metabolism shifts from starch accumulation to soluble sugars accumulation (4 MAFB treatment). In this period, continuous accumulation of dry matter by the fruit while starch is hydrolyzing and soluble solids are increasing, indicates that a continuous photoassimilate import to the fruits occurs (Okuse and Ryugo, 1981). Thus, the obtained results suggest that ABA enhances soluble sugar accumulation by either enhancing starch hydrolization or by increasing photoassimilate unloading to the fruits. In fact, several reports indicate that ABA boosts carrier-mediated sugar uptake in strawberry (Ofosu-Anim et al., 1996), melon (Ofosu-Anim et al., 1994), apple (Peng et al., 2003) and peach (Kobashi et al., 2001), and enhances sucrose and sorbitol cleavage enzyme activity in peach (Kobashi et al., 1999), grape (Pan et al., 2005) and apple (Pan et al., 2006). In contrast, ABA produces a ripening delay (2 MAFB treatment) when fruit sugar metabolism shifts from glucose to starch accumulation suggesting that the hormone negatively affects the onset of starch synthesis and thus delays soluble solids accumulation at harvest. Similar results were obtained in kiwifruit by vine-heating in summer and prior to harvest; in the former fruit DM content was greatly reduced (Richardson et al., 2004) and, on the other hand, vegetative vigor was enhanced;
in the latter, DM increased (Snelgar et al., 2005). It is possible that summer treatments (2 MAFB) negatively affect growth and ripening by favouring other actively growing organs, such as shoots.

In late treatments, differences in SSC between 2009 and 2010 seasons could be explained because 2009 harvests were delayed due to environmental conditions and thus ripening of both treated and control fruits equalized in the tree. In the present study, fruit ripening was differently affected during storage by ABA treatments. Fruits of early treatments (1 and 2 MAFB) exhibited the same differences after the storage period as at harvest time. On the contrary, ABA-treated fruits of later applications (3 and 4 MAFB) showed a delayed ripening pattern as compared to their respective controls, since the increment rate of SSC and DM was similar or lower in treated fruits.

4.3.2 Field PDJ and ABA applications improve quality of Actinidia chinensis fruits.

At harvest, ABA- and PDJ-treated fruits showed altered quality traits as compared to control fruits (Table 4.14 to 4.16). Flesh color development of yellow kiwifruits was enhanced by PDJ treatments at commercial harvest in both years, as indicated by a reduction in H° and flesh IAD; ABA applications instead, determined similar or higher values of H° and flesh IAD at commercial harvest. In the 2009 delayed harvest, there were no color differences between treatments. As far as sugar accumulation is concerned, both treatments increased it towards harvest. In the first season (both harvests), ABA-treated fruits showed the highest accumulation of soluble solids, PDJ-treated fruits exhibited intermediate amounts, and control ones had the lowest content; in the second season, PDJ and ABA-treated fruits had higher SSC levels than control ones. Moreover, PDJ treatments significantly increased DM content and ABA-treated fruits showed higher IAD at harvest in 2010. In both years FF, TA and fresh weight (data not shown for TA and weight) were unaffected by the treatments.
ABA and PDJ treatments during fruit development improve Kiwifruit quality

Table 4.14 Effects of 2009 pre-harvest ABA and PDJ treatments on kiwifruit quality traits at commercial harvest

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Skin I&lt;sub&gt;AD&lt;/sub&gt;</th>
<th>Flesh I&lt;sub&gt;AD&lt;/sub&gt;</th>
<th>Hue angle (H°)</th>
<th>FF (kg cm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>SSC (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.68 a</td>
<td>0.35 ab</td>
<td>102 a</td>
<td>4.15 a</td>
<td>11.6 b</td>
</tr>
<tr>
<td>ABA</td>
<td>1.63 a</td>
<td>0.39 a</td>
<td>103 a</td>
<td>4.52 a</td>
<td>12.4 a</td>
</tr>
<tr>
<td>PDJ</td>
<td>1.70 a</td>
<td>0.19 b</td>
<td>100 b</td>
<td>4.29 a</td>
<td>12.1 ab</td>
</tr>
</tbody>
</table>

Significance: n.s., not significant; *, significant difference at P ≤ 0.05; **, P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 4.15 Effects of 2009 pre-harvest ABA and PDJ treatments on kiwifruit quality traits at 1-week delayed harvest.

| Treatments | Skin I<sub>AD</sub> | Flesh I<sub>AD</sub> | Hue angle (H°) | FF (kg cm<sup>3</sup>) | SSC (°Brix) | DM (%) |
|------------|----------------------|----------------------|-----------------|------------------------|------------|
| Control    | 1.62 a               | 0.24 a               | 99 a            | 3.92 a                 | 13.6 b     | 20.6 a |
| ABA        | 1.65 a               | 0.20 a               | 98 a            | 3.56 a                 | 14.3 a     | 20.6 a |
| PDJ        | 1.64 a               | 0.17 a               | 98 a            | 3.94 a                 | 14.2 ab    | 20.5 a |

Significance: n.s., not significant; **, significant difference at P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 4.16 Effects of 2010 pre-harvest ABA and PDJ treatments on kiwifruit quality traits at commercial harvest.

| Treatments | Skin I<sub>AD</sub> | Flesh I<sub>AD</sub> | Hue angle (H°) | FF (kg cm<sup>3</sup>) | SSC (°Brix) | DM (%) |
|------------|----------------------|----------------------|-----------------|------------------------|------------|
| Control    | 1.25 b               | 0.16 ab              | 101 a           | 5.69 a                 | 8.6 b      | 18.5 a |
| ABA        | 1.39 a               | 0.23 a               | 101 a           | 5.88 a                 | 9.8 a      | 18.4 a |
| PDJ        | 1.15 b               | 0.09 b               | 99 b            | 5.92 a                 | 10.5 a     | 19.6 b |

Significance: **, *, n.s., not significant; *, significant difference at P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

After cold storage (Tables 4.17 and 4.18), treated fruits from both seasons presented differences in their quality attributes. In the first season, PDJ-treated fruits showed lower values of H° and flesh I<sub>AD</sub> as compared to control ones; ABA treatments produced fruits with higher FF
and intermediate flesh $I_{AD}$ as compared to controls the first season, and reduced FF the second season. No differences were found in skin $I_{AD}$, TA or DM in both seasons among treatments.

**Table 4.17** Effects of pre-harvest ABA and PDJ treatments (2009) on kiwifruit quality traits after 2 month storage at 1°C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Skin $I_{AD}$</th>
<th>Flesh $I_{AD}$</th>
<th>Hue angle (H)</th>
<th>FF (kg cm$^{-3}$)</th>
<th>SSC (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.33 a</td>
<td>0.033 a</td>
<td>96.6 a</td>
<td>1.39 b</td>
<td>16.7 a</td>
</tr>
<tr>
<td>ABA</td>
<td>1.31 a</td>
<td>0.023 ab</td>
<td>96.6 a</td>
<td>1.55 a</td>
<td>16.4 a</td>
</tr>
<tr>
<td>PDJ</td>
<td>1.25 a</td>
<td>0.006 b</td>
<td>95.7 b</td>
<td>1.34 b</td>
<td>16.8 a</td>
</tr>
</tbody>
</table>

Significance: n.s., not significant; *, significant difference at $P \leq 0.05$; **, $P \leq 0.01$. Data represent mean values. In each column, means followed by the same letter are not statistically different (at $P \leq 0.05$).

**Table 4.18** Effects of pre-harvest ABA and PDJ treatments (2010) on kiwifruit quality traits after 2 month storage at 1°C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Skin $I_{AD}$</th>
<th>Flesh $I_{AD}$</th>
<th>Hue angle (H)</th>
<th>FF (kg cm$^{-3}$)</th>
<th>SSC (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.12 a</td>
<td>0.03 a</td>
<td>99.4 a</td>
<td>0.75 a</td>
<td>16.5 a</td>
</tr>
<tr>
<td>ABA</td>
<td>1.13 a</td>
<td>0.02 a</td>
<td>99.5 a</td>
<td>0.48 b</td>
<td>16.6 a</td>
</tr>
<tr>
<td>PDJ</td>
<td>1.13 a</td>
<td>0.02 a</td>
<td>99.0 a</td>
<td>0.69 a</td>
<td>16.2 a</td>
</tr>
</tbody>
</table>

Significance: n.s., not significant; ***, significant difference at $P \leq 0.001$. Data represent mean values. In each column, means followed by the same letter are not statistically different (at $P \leq 0.05$).

The relationship between external and flesh $I_{AD}$ and flesh color and dry matter content of ‘Jintao’ fruits at harvest is exposed in Figure 4.1 and 4.2. In the 2009 season, $H^\circ$ positively correlated with flesh $I_{AD}$ with a correlation coefficient ($r$) of 0.7967 for the commercial harvest and $r$ of 0.7048 for the delayed harvest. In the 2010 season, both maturity indexes positively correlated between them ($r = 0.757$) and $H^\circ$, and negatively with DM. These trends suggest that fruits with lower $I_{AD}$ show lower $H^\circ$ and high DM and are riper.
Present data show that ABA and PDJ treatments enhanced fruit ripening by enhancing SSC whereas color development was only enhanced by PDJ. Several reports indicate that exogenous JA and ABA treatments modify ripening-related parameters in diverse fruit species, especially color development by stimulating either anthocyanin synthesis and/or chlorophyll degradation.
ABA and PDJ treatments during fruit development improve Kiwifruit quality

(Kondo et al., 2001; Kondo et al., 2002; Rudell et al., 2002; Jiang and Joyce, 2003; Jeong et al., 2004; Wang et al., 2005; Wang and Zheng, 2005; Rudell et al., 2005; Peppi et al., 2006; Wang et al., 2007; Peppi and Fidelibus, 2008; Peppi et al., 2008); other ripening related parameters such as fruit FF and SSC, may be unaltered or differentially affected (Kondo and Gemma, 1993; Kojima et al., 1995; Fan et al., 1998b; Gonzalez-Aguilar et al., 2004; Kondo et al., 2005; Wang and Zheng., 2005; Wang et al., 2007; Peppi and Fidelibus, 2008; Ziosi et al., 2008a). In A. chinensis fruits, during the progression of maturation, chlorophylls eventually disappear completely whereas no major differences in carotenoid content and composition occur as compared with Actinidia deliciosa fruits (McGhie and Ainge, 2002; Nishiyama et al., 2008; Montefiori et al., 2009); this suggests that improved color development by PDJ treatments is due to increased chlorophyll degradation rather than changes in carotenoid content in accord with the known de-greening effect of JAs (Tsuchiya et al., 1999; Wang et al., 2005).

Also, both treatments increased SSC when applied late in the season. Actinidia chinensis fruits follow a similar trend in sugar metabolism as Actinidia deliciosa (Boldingh et al., 2000), thus, according to point 4.3.1, this increase may be due to either enhanced starch hydrolization or increased photoassimilate unloading. Several reports regard the involvement of ABA in sugar metabolism whereas the mechanism by which JAs might affect fruit sugar accumulation is not clear. However, there are reports indicating increased or decreased SSC after pre- and post-harvest JA treatments (Wang and Zheng, 2005; Rohwer and Erwin, 2008; Ziosi et al., 2008a).

In this study, both destructive and non-destructive kiwi-meter measurements showed good correlations with internal flesh color in 2010 harvest whereas in 2009 only flesh IAD correlated with flesh color. Studies indicate that excessive sun exposure leads to changes in fruit skin morphology, wax composition and structure (Woolf and Ferguson, 2000), thus reducing light penetration and external IAD accuracy. Present data suggests that IAD can replace traditional flesh color determinations in fruits from well-covered vines. Also, in 2010 in A. chinensis both IAD correlated very well with fruit DM content which is interesting though DM is less important than in A. deliciosa in quality determination of yellow kiwifruit.
4.3.3 Post-harvest PDJ and ABA dipping affects kiwifruit quality traits after storage.

The effects of post-harvest ABA and/or PDJ dipping on yellow kiwifruit quality traits are displayed on Table 4.19. SSC was higher in fruit treated with the higher ABA concentration (200 ppm) and in fruits treated with ABA + PDJ as compared with controls. Softening was reduced by 20 ppm PDJ treatments and color was enhanced by the combination ABA + PDJ. The rest of the treatments resulted in similar values for all the parameters as compared with controls.

Table 4.19 Effects of post-harvest ABA and PDJ treatments on kiwifruit quality traits after 2 months of cold storage. 2009

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Skin IAD</th>
<th>Hue angle (H)</th>
<th>Flesh IAD</th>
<th>FF (kg cm⁻³)</th>
<th>SSC (°Brix)</th>
<th>TA (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.28 ab</td>
<td>96.6 a</td>
<td>0.02 abc</td>
<td>1.10 b</td>
<td>16.2 c</td>
<td>8.63 a</td>
</tr>
<tr>
<td>ABA 20 ppm</td>
<td>1.31 ab</td>
<td>96.2 ab</td>
<td>0.03 ab</td>
<td>1.19 ab</td>
<td>16.5 bc</td>
<td>9.97 a</td>
</tr>
<tr>
<td>ABA 200 ppm</td>
<td>1.23 b</td>
<td>96.0 ab</td>
<td>0.00 c</td>
<td>1.22 ab</td>
<td>16.9 b</td>
<td>9.50 a</td>
</tr>
<tr>
<td>PDJ 20 ppm</td>
<td>1.32 a</td>
<td>96.1 ab</td>
<td>0.01 bc</td>
<td>1.30 a</td>
<td>16.5 bc</td>
<td>9.90 a</td>
</tr>
<tr>
<td>PDJ 200 ppm</td>
<td>1.22 b</td>
<td>96.7 a</td>
<td>0.04 a</td>
<td>1.18 b</td>
<td>16.0 c</td>
<td>9.97 a</td>
</tr>
<tr>
<td>ABA 20 + PDJ 20</td>
<td>1.34 a</td>
<td>95.7 b</td>
<td>0.02 abc</td>
<td>1.22 ab</td>
<td>17.6 a</td>
<td>8.83 a</td>
</tr>
</tbody>
</table>

Significance: * n.s., not significant; *, significant difference at P ≤ 0.05; ***, P ≤ 0.001. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Present study shows that exogenous ABA and PDJ altered some quality parameters, such as SSC and FF, during cold storage. PDJ treatments at low dose reduced softening, exogenous ABA at the higher concentration enhanced SSC, whereas both plant growth regulators supplied together improved SSC more than ABA alone. Diverse results have been reported concerning JA and ABA effects on ripening-related parameters (refer to point 4.3.2). In nectarines, MJ and PDJ field applications reduce ethylene emission, softening and color development depending on the fruit physiological stage (Ziosi et al., 2008a). This study suggests that post-harvest ABA and PDJ treatments do not greatly alter kiwifruit quality parameters during cold storage, probably because fruits were already ripe and yellow when treated. It is possible that the treatment with either hormone of less ripe fruits than those presently used, which still have a green flesh, could accelerate flesh color development as demonstrated in apples (Kondo et al., 2001; Rudell et al., 2002) and Actinidia macrosperma (Montefiori et al., 2007) after postharvest JA treatments, and
in litchi (Wang et al., 2007), grape (Peppi et al., 2006; Peppi et al., 2007) and strawberry (Jiang and Joyce, 2003) for postharvest ABA treatments. It is not clear how JAs and ABA interacts during fruit ripening; in apples exogenous JA treatments to preclimacteric and climacteric fruits reduce ABA accumulation whereas exogenous ABA enhances JA accumulation in all kinds of fruits, via an increase in ethylene production (Kondo et al., 2001). In this case it seems that low doses of PDJ and ABA act in a synergistic way to enhance ripening.

### 4.3.4 Field ABA application reduces gas exchanges

In both trials, exogenous ABA treatment induced a reduction in gas exchange parameters. In girdled branches (Table 4.20) significant differences occurred 1 day after treatment. ABA-treated branches showed a 20-30% reduction in the levels of A, gs, and E. In full vine treatments (Table 4.21), gas exchange alterations started the same day of the treatment and lasted for 1 day. In this trial, ABA-treated plants showed a 10-20% reduction in the levels of A, gs and E. In both treatments instantaneous WUE was not significantly altered.

**Table 4.20** Gas exchange measurements after ABA treatments to girdled branches.

<table>
<thead>
<tr>
<th>Assessment date</th>
<th>Treatment</th>
<th>Assessment date</th>
<th>Treatment</th>
<th>A (µmol m⁻² s⁻¹)</th>
<th>gs (mol m⁻² s⁻¹)</th>
<th>E (mmol m⁻² s⁻¹)</th>
<th>WUEi (µmol mmol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same Day</td>
<td>Control</td>
<td>6.89 a</td>
<td>0.14 a</td>
<td>2.43 a</td>
<td>2.85 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>6.87 a</td>
<td>0.13 a</td>
<td>2.27 a</td>
<td>3.04 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DAT</td>
<td>Control</td>
<td>6.95 a</td>
<td>0.17 a</td>
<td>4.07 a</td>
<td>1.70 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>5.21 b</td>
<td>0.12 b</td>
<td>3.12 b</td>
<td>1.65 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.s., not significant; ***, significant difference at P ≤ 0.001. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
Table 4.21 Gas exchange measurements after ABA treatments to full vines.

<table>
<thead>
<tr>
<th>Assessment date</th>
<th>Treatment</th>
<th>A (µmol m⁻² s⁻¹)</th>
<th>gₛ (mol m⁻² s⁻¹)</th>
<th>E (mmol m⁻² s⁻¹)</th>
<th>WUEi (µmol mmol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same Day</td>
<td>Control</td>
<td>12.4. a</td>
<td>0.26 a</td>
<td>6.99 a</td>
<td>1.78 a</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>11.2 b</td>
<td>0.24 a</td>
<td>6.47 a</td>
<td>1.67 a</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td></td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>1 DAT</td>
<td>Control</td>
<td>11.1 a</td>
<td>0.25 a</td>
<td>6.45 a</td>
<td>1.74 a</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>9.1 b</td>
<td>0.19 b</td>
<td>5.28 b</td>
<td>1.72 a</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td></td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>2 DAT</td>
<td>Control</td>
<td>7.78 a</td>
<td>0.16 a</td>
<td>5.17 a</td>
<td>1.51 a</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>8.63 a</td>
<td>0.176 a</td>
<td>5.65 a</td>
<td>1.52 a</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

It is broadly known that ABA plays a central role in plant-water relations as it induces stomatal closure and prevents stomata opening, and hence it reduces plant gas exchange (Chaves, 1991; McAdams et al., 2011). In the present work, reduction in gas exchange parameters suggests that supplied ABA was taken up by the leaves. In fact, exogenously applied ABA induces stomatal closure in several species such as wheat (Quarrie and Jones, 1977) and *Malus sp.* (Ma et al., 2008). The transient reduction in gas exchange exhibited in full vines can be due to fact that kiwifruit does not acclimate to dry climate. Acclimated species show a better responsiveness to exogenous ABA (Li et al., 2004a; Ma et al., 2008).

In conclusion, both hormone treatments seem to regulate some aspects of kiwifruit ripening with particular emphasis on SSC (ABA and PDJ) and flesh color (PDJ). The possibility of the combined application of the two hormones is to be considered though further investigation is needed.
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

5. ABA TREATMENTS REDUCE WATER USE IN PEACH PLANTS, AND PEACH AND NECTARINE DETACHED BRANCHES

5.1 Introduction

Abscisic acid (ABA), a well-known plant hormone, first discovered in the 1960’s under the names of either abscissin or dormin in young cotton fruits and in dormant buds of sycamore, plays key roles in seed and organ dormancy, plant responses to biotic and abiotic stress and sugar sensing (Schwartz and Zeevaart, 2010). ABA biosynthesis requires the cleavage of C\textsubscript{40} carotenoids to form its direct precursor (Nambara and Marion-Poll, 2005). This process, catalyzed by the 9-cis-epoxycarotenoid dehydrogenase enzyme (NCED), is the main rate-limiting step in ABA biosynthesis; in fact, alterations in NCED activity in deficient maize and over-expressing tomato mutants lead to reduced or enhanced ABA accumulation, respectively (Tan \textit{et al.}, 1997; Thompson \textit{et al.}, 2007). NCED over-expressing plants present enhanced ABA accumulation, drought tolerance and reduced transpiration (Iuchi \textit{et al.}, 2001; Qin and Zeevaart, 2002; Thompson \textit{et al.}, 2007)

Water availability is one of the major factors that determine crop yield and plant growth (Iuchi \textit{et al.}, 2001). Numerous studies demonstrated that cell expansion and growth decline under water deficit while progressive water deficit negatively affects photosynthesis and carbon partitioning (Chaves \textit{et al.}, 2002; Taiz and Zeiger, 2010). ABA differentially affects root and shoot growth under diverse water conditions. At high water potential shoot growth, and root growth to a lesser extent, is greater in wild-type maize plants than in ABA-deficient mutants. In contrast, limiting water availability determines opposite effects on shoots and roots; shoot growth is greater in ABA-deficient mutants whereas root growth is higher in wild-type plants (Sharp amnd LeNoble, 2002). In \textit{Malus} and \textit{Populus} plants, drought conditions reduce total biomass production and increase the root to shoot relation (Li \textit{et al.}, 2004a; Ma \textit{et al.}, 2008). Exogenous ABA treatments inhibit plant growth under normal growth conditions and enhance it under stress conditions (Khadri \textit{et al.}, 2006).

In the present work, peach was chosen as a model to shed some light on the physiological role of ABA on water relations and plant growth, under diverse water availability conditions. Exogenous ABA was applied to potted peach plants and detached nectarine and peach branches
and the following were analysed: (i) transpiration, (ii) biomass production, and (iii) fruit dehydration.

5.2 Materials and Methods

5.2.1 Plant material and experimental design

Micro-propagated plants

The trial was carried out with micro-propagated peach rootstock ‘GF677’ plants, potted in 2 l containers with a potting peat substrate, and kept under the desired pot water conditions in a greenhouse. After plant adaptation to the greenhouse conditions and water regimes, they were treated with ABA through soil drenching or leaf sprays (Table 5.1). Pot water conditions were established as a percentage of bulk water (BW) held by the pots. BW was calculated by subtracting from saturated pot weight (after they were flooded and left to percolate during the night) the dry pot weight (after drying the substrate). After treatment, plants were grown for 4 weeks and water use was assessed daily. Finally, at the end of the trial, all the plants were partitioned in roots and shoots to assess plant biomass production; water use efficiency, as biomass produced per water transpired, was also determined.

<table>
<thead>
<tr>
<th>Pot water conditions</th>
<th>Treatment</th>
<th>Replicate number</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 % BW</td>
<td>50 ppm ABA drench</td>
<td>4 plants</td>
</tr>
<tr>
<td></td>
<td>500 ppm ABA spray</td>
<td>4 plants</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>4 plants</td>
</tr>
<tr>
<td>50 % BW</td>
<td>50 ppm ABA drench</td>
<td>4 plants</td>
</tr>
<tr>
<td></td>
<td>500 ppm ABA spray</td>
<td>4 plants</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>4 plants</td>
</tr>
<tr>
<td>No irrigation</td>
<td>50 ppm ABA drench</td>
<td>4 plants</td>
</tr>
<tr>
<td></td>
<td>500 ppm ABA spray</td>
<td>4 plants</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>4 plants</td>
</tr>
</tbody>
</table>
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

Detached peach branch model

One year old detached ‘Flaminia’ peach and ‘Stark Red Gold’ nectarine branches, composed by one shoot and one fruit, were harvested at different stages of fruit development from seven year-old peach trees (*Prunus persica* L. Batsch) and fifteen-year-old nectarine trees (*Prunus persica* L. Batsch) grown at a commercial orchard in Faenza and at the S. Anna experimental station of the university of Bologna, Italy, respectively. To identify fruit development stages, a growth curve was established for both cultivars (Fig. 5.1 and 5.2). To ensure branch uniformity, twig selection was carried out by taking limbs with an active growing shoot and a fruit with a known DA-value. After picking, branches were immediately taken to a growth chamber, placed in 50 ml graduated falcon tubes filled with water, or with an abscisic acid (ABA; S-(-)-abscisic acid; Valent Biosciences, Libertyville, IL, USA) solution and left to grow for 5 or 6 days (nectarine and peach, respectively) under controlled conditions (24°C, and 14/10 day/night cycles). Flaminia branches were also sprayed with an ABA solution. Treatments are listed in tables 5.1 and 5.2.

![Graph showing fruit diameter and DA index over time](image)

**Figure 5.1** ‘Stark Red Gold’ nectarine growth curve and DA-index evolution in time. Arrows indicate branch harvest time.
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

Figure 5.2 ‘Flaminia’ peach growth curve. The arrow indicates branch harvest date.

Table 5.1 ‘Stark Red Gold’ detached branch treatments.

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>IAD-value</th>
<th>Treatments</th>
<th>Replicate number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early S3</td>
<td>2.2 – 2.0</td>
<td>5 ppm ABA dipping</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>24</td>
</tr>
<tr>
<td>Mid S3</td>
<td>2.0 – 1.8</td>
<td>5 ppm ABA dipping</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>25</td>
</tr>
<tr>
<td>S3/S4 transition</td>
<td>1.6 – 1.4</td>
<td>5 ppm ABA dipping</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>15</td>
</tr>
<tr>
<td>S4</td>
<td>1.0 – 0.8</td>
<td>5 ppm ABA dipping</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 5.2 ‘Flaminia’ detached branch treatments.

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>IAD-value</th>
<th>Treatments</th>
<th>Replicate number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early S3</td>
<td>2.2 – 2.0</td>
<td>5 ppm ABA dipping</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 ppm ABA foliar spray</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>9</td>
</tr>
</tbody>
</table>

For both cultivars, daily branch water use was assessed until branch senescence and fruit dehydration was determined during the early S3 treatments.
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

5.2.2 Water use

Detached branch water use (WU) was assessed by daily measuring differences in water or solution levels in the tube. After each assessment tubes were refilled when needed with water or ABA solution respectively.

WU of micro-propagated plants was measured through periodic pot weighting with a scale. Pots were refilled, either with water or ABA solution, after each measurement to maintain the desired water conditions. For both experimental designs WU was expressed as accumulated transpiration throughout the trial period.

5.2.3 Fruit dehydration

Fruit dehydration was qualitatively evaluated 4 and 5 DAT following the subsequent scheme:

<table>
<thead>
<tr>
<th>Fruit dehydration</th>
<th>Dehydration mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>No visual signs of dehydration</td>
<td>0</td>
</tr>
<tr>
<td>Initial dehydration signs</td>
<td>1</td>
</tr>
<tr>
<td>Evident visual dehydration</td>
<td>2</td>
</tr>
<tr>
<td>Severe visual dehydration</td>
<td>3</td>
</tr>
</tbody>
</table>

5.2.4 Biomass production

To determine biomass production, all plants were destroyed at the end of the experiment (four weeks after the treatments), divided in shoots and roots, and oven-dried at 65°C for 4 days and weighted.

5.2.5 Branch uniformity

Nectarine and peach branch selection was performed on the basis of fruit ripening homogeneity as determined by the DA-meter (Refer to point 2.2.2).
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

5.2.6 Statistical analysis

All data were statistically analyzed using a completely randomized design. In ‘Stark Red Gold’ detached branches, treatment (2 levels: ABA and control) and fruit development stage (4 levels: early-S3, mid-S3, S3/S4 and S4) were considered as factors; in ‘Flaminia’ detached branches the factor was the treatment (3 levels: control, spray and dipping). As to the micro-propagated plant trial, the factors used were treatment (3 levels: control, spray and drench), and pot water condition (3 levels: no-irrigation, 50 and 100 % BW).

5.3 Results and Discussion

5.3.1 ABA reduces transpiration in micro-propagated peach plants, and peach and nectarine detached branches

Micro-propagated plants

In micro-propagated plants, ABA treatments differentially affected WU depending on pot water conditions. Under non-irrigated conditions (Fig. 5.3a), ABA treatments transiently reduced plant transpiration from 2 to 13 DAT, but there were no significant differences in total WU at the end of the period, suggesting a faster depletion of water reserves by control plants. Under water stressed conditions (50% BW; Fig. 5.3b), both ABA treatments decreased WU from 2 DAT onwards as compared to controls; this reduction accounted for ~50% and ~20% at the end of the period when ABA was supplied by drench or spray, respectively. Finally, under optimal irrigation conditions (100% BW; Fig. 5.3c), drench-treated plants showed reduced WU throughout the experiment, whereas sprayed-plants had significantly lower WU from 2 to 19 DAT; at the end of the experiment WU of drench-treated and spray-treated plants was lower than that of control ones (~50% and ~75% of control ones, respectively).
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

Figure 5.3 Accumulated water use in micro-propagated GF677 plants after ABA treatments. a, after 4 weeks without irrigation, b, at 50% BW and c, at 100% BW respectively.* significant differences at P ≤ 0.05; ** at P ≤ 0.01; *** at P ≤ 0.001. Data represent mean values ± standar error.
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

Detached branches

In ‘Stark Red Gold’ nectarines, daily WU followed a decreasing trend, throughout the period, in control and treated branches (Fig. 5.4a), with ABA-treated ones transpiring significantly less water (~35% at each evaluation date) than controls throughout the period. As far as the fruit growth stage is concerned (Fig. 5.4b), daily WU also had a decreasing trend at all evaluated stages, with S4 branches using significantly more water than the other ones during the trial period: from ~35% to ~300% more than early S3 ones, ~30% to ~270% more than mid-S3 ones, and ~20% to ~65% more than S3/S4 ones.

Accumulated WU followed an increasing trend, throughout the period, in control and treated branches (Fig. 5.5a), with ABA-treated ones transpiring significantly less water than controls throughout the period; at the end of the period, treated branches transpired an average of 35% less water than controls with early S3, mid-S3, S3/S4 and S4 treatments exhibiting a reduced branch transpiration by 50, 34, 26 and 26 % as compared with controls, respectively. As far as the fruit growth stage is concerned (Fig. 5.5b), accumulated WU also showed an increasing trend at all evaluated stages, with S4 branches using significantly more water than the others during the trial period, followed by S3/S4, mid-S3 and early S3 ones. At the end of the period, early S3, mid-S3 and S3/S4 branches transpired ~58%, ~66% and ~85% of the water transpired by S4 ones, respectively.

Figure 5.4 Nectarine detached branch daily water use as influenced by: a, ABA treatment; b, Fruit growth stage. *, significant differences at P ≤ 0.05; **, at P ≤ 0.01; ***, at P ≤ 0.001. Data represent mean values.
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

Figure 5.5 Nectarine detached branch accumulated water use as influenced by: a, ABA treatment; b, Fruit growth stage. *, significant differences at P ≤ 0.05; **, at P ≤ 0.01; ***, at P ≤ 0.001. Data represent mean values.

In peach detached branches, daily WU also followed a decreasing trend through the period, for all treatments (Fig. 5.6a), with spray-treated branches transpiring significantly less water than controls throughout the period (from ~45 to ~70% at each evaluation time), and dipping-treated ones transpiring less than controls at 1 and 6 DAT (from ~30 to ~40%). Accumulated WU followed an increasing trend through the period, in all treatments (Fig. 5.6b), with ABA-treated branches transpiring significantly less water than controls throughout; at the end of the period, sprayed and dipping-treated branches transpired ~40% and ~30% less water than controls. No significant differences were found between ABA application modes (sprayed or dipping).

Figure 5.6 Peach detached branch water use after ABA treatments. a, daily water use; b, accumulated water use. *, significant differences at P ≤ 0.05; **, at P ≤ 0.01; ***, at P ≤ 0.001. Data represent mean values.
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

It has been demonstrated that ABA plays a key role in stomatal movements, and in short- and long-term responses to water deficit (Chaves et al., 2002) thus influencing plant water relationships. Present data show that exogenous ABA treatments reduce plant transpiration under both well-watered and drought conditions. In micro-propagated plants, transpiration under well-watered conditions for both ABA treatments was similar compared with control plants under drought conditions suggesting that ABA mimics plant responses to water shortage. Similar results were found for Populus davidiana (Li et al., 2004a) and Malus sp. (Ma et al., 2008) in which periodically applied ABA reduced plant gas exchange (net photosynthesis, transpiration and stomatal conductance) and increased WUE under both well-watered and drought conditions. Exogenous ABA effect was higher when the hormone was applied directly with the irrigation water than when it was sprayed on the leaves; this may be due to the fact that, in the former, ABA was supplied continuously while in the latter ABA sprays were done only once at the beginning of the trial. Sprayed ABA-treatments, under both well-watered and drought conditions, showed transient reduction in plant transpiration that lasted until ~15 days after treatment; afterwards daily water transpiration returned to control levels. However, the initial reduction in transpiration was enough to significantly reduce total water used after ~1 month of evaluations. In non-irrigated plants, ABA treatments had no effect on total water used; however, they reduced initial plant transpiration and delayed water reserve depletion thus increasing plant tolerance to progressive drought.

In detached branches, as fruit development progressed, WU increased while ABA-effect on total water loss decreased. The increased transpiration can be explained by the increasing fruit water loss and xylem water inflow with advancing fruit development that characterize peach (Morandi et al., 2007); detached branch leaf area remained uniform among the different stages analyzed. This data suggest that ABA mainly acts on leaf transpiration and does not interfere with fruit transpiration; thus, it should not produce detrimental effects on fruits growth which in peaches is maintained by high evaporative rates that facilitate phloem unloading and cell elongation. In contrast with micro-propagated plants, no differences were found in the decreasing WU of detached branches between spray and dipping ABA treatments, possibly due to the short duration of the trial. However, it should be noted that ABA catabolism rapidly occurs in plants (Jia et al., 1996; Ren et al., 2007) so that periodically supplying ABA should lead to better results than one-time treatments.
5.3.2 ABA reduces fruit dehydration in detached branches

In both cultivars, fruit dehydration tended to increase with time (Table 5.4 and 5.5). In nectarine detached branches, the progression of fruit desiccation was reduced by ABA treatment, and remained lower at the end of the trial period. In peach twigs, only dipping-applied ABA reduced dehydration progression as compared to controls while sprayed branches exhibited intermediate levels of dehydration at the end of the trial period.

Table 5.4 ‘Stark Red Gold’ fruit dehydration after ABA treatments done during S3 stage of fruit growth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 DAT</th>
<th>4 DAT</th>
<th>5 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.79 a</td>
<td>1.71 a</td>
<td>1.86 a</td>
</tr>
<tr>
<td>Treated</td>
<td>0.00 b</td>
<td>0.21 b</td>
<td>0.71 b</td>
</tr>
<tr>
<td>significance</td>
<td>*</td>
<td>**</td>
<td>*</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at P ≤ 0.05; **, P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

Table 5.5 ‘Flaminia’ fruit dehydration after ABA treatments done during S3 stage of fruit growth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 DAT</th>
<th>5 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.71 a</td>
<td>1.71 a</td>
</tr>
<tr>
<td>Dipping</td>
<td>0.22 b</td>
<td>0.56 b</td>
</tr>
<tr>
<td>Spray</td>
<td>1.14 a</td>
<td>1.20 ab</td>
</tr>
<tr>
<td>significance</td>
<td>**</td>
<td>*</td>
</tr>
</tbody>
</table>

n.s., not significant; *, significant difference at P ≤ 0.05; **, P ≤ 0.01. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).

In peaches, daily water losses through skin transpiration account for 50% of the imported water during S1 and S3 growth stages (Morandi et al., 2007). This loss induces a decrease in fruit water potential and an increase in phloem imports thus enhancing fruit fresh weight and dry matter gain (Morandi et al., 2010). Present data show that fruit dehydration was significantly reduced by ABA treatments only when applied by dipping; this suggests that total water influx in treated fruits was higher than in control fruits and, thus, treated fruits handled better with skin transpiration. This difference in performance between ABA treatments could be due to the
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

constant ABA flow during dipping that enhances water and solute transport towards developing fruits.

5.3.3 ABA does not modify plant growth patterns under different water conditions

The effects of ABA treatment on micro-propagated plant growth are listed in Table 5.6 to 5.8. ABA treatments did not induce any significant effect in single organs or in total biomass production under different pot water conditions while WUE was enhanced by ABA drench treatment under drought conditions. However, ABA treatments tended to decrease shoot, root to shoot ratio (R/S) and total biomass production as compared to control plants in well-watered conditions. In drought conditions, drench ABA treatments slightly reduce root, shoot and canopy biomass accumulation as compared to both spray-treated and control plants. Finally, under non-irrigation conditions ABA increases shoot and total biomass production and reduce R/S ratio as related to control and spray-treated plants. Regarding the effect of water availability on plant growth (data not shown), non-irrigated plants produced significantly less root, shoot and total biomass than drought and well-watered plants; the latters produced similar root, shoot and total biomass. Also, WUE decreased with increasing water availability.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root (g DW)</th>
<th>Shoot (g DW)</th>
<th>R/S</th>
<th>Total (g DW)</th>
<th>WUE (g DW g⁻¹ H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.62 a</td>
<td>3.75 a</td>
<td>0.17 a</td>
<td>4.37 a</td>
<td>1.90 a</td>
</tr>
<tr>
<td>Drench</td>
<td>0.66 a</td>
<td>2.73 a</td>
<td>0.26 a</td>
<td>3.39 a</td>
<td>2.05 a</td>
</tr>
<tr>
<td>Spray</td>
<td>0.75 a</td>
<td>2.95 a</td>
<td>0.26 a</td>
<td>3.70 a</td>
<td>1.95 a</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).
Endogenous ABA, besides controlling stomatal movements, alters plant growth upon soil environmental conditions by sustaining growth under normal conditions and enhancing it under limiting conditions such as drought (Sharp, 2002; LeNoble et al., 2004). Previous reports demonstrated that periodically supplied ABA increases WUE and reduces plant growth under well-watered and drought conditions in *Populus davidiana* (Li et al., 2004a) and two diverse *Malus* species (Ma et al., 2008) due to a rise in endogenous ABA content. Present data show that exogenous ABA treatments produced no significant effect on plant growth under different water availability conditions and only increased WUE under drought conditions. In *Populus davidiana* and *Malus* sp., well-watered plants produced more biomass than drought stressed ones (Li et al., 2004a; Ma et al., 2008). In contrast, in the present study, both well-watered and drought-stressed plants produced similar total biomass whereas non-irrigated plants showed the lowest biomass production. This difference compared with previous experiments may be explained by the study duration, which extended for about 1 month in the present work; in fact, *Populus davidiana* (Li et al., 2004a) and *Malus* (Ma et al., 2008) studies prolonged for 4 and 6 months, respectively. Also,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root (g DW)</th>
<th>Shoot (g DW)</th>
<th>R/S</th>
<th>Total (g DW)</th>
<th>WUE (g DW g⁻¹ H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69 a</td>
<td>3.15 a</td>
<td>0.21 a</td>
<td>3.84 a</td>
<td>2.22 b</td>
</tr>
<tr>
<td>Drench</td>
<td>0.59 a</td>
<td>2.56 a</td>
<td>0.23 a</td>
<td>3.15 a</td>
<td>3.56 a</td>
</tr>
<tr>
<td>Spray</td>
<td>0.61 a</td>
<td>3.03 a</td>
<td>0.20 a</td>
<td>3.64 a</td>
<td>2.57 b</td>
</tr>
</tbody>
</table>

*Significance n.s., not significant; *, significant difference at P ≤ 0.05. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root (g DW)</th>
<th>Shoot (g DW)</th>
<th>R/S</th>
<th>Total (g DW)</th>
<th>WUE (g DW g⁻¹ H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.47 a</td>
<td>2.07 a</td>
<td>0.23 a</td>
<td>2.54 a</td>
<td>3.87 a</td>
</tr>
<tr>
<td>Drench</td>
<td>0.49 a</td>
<td>2.50 a</td>
<td>0.20 a</td>
<td>3.00 a</td>
<td>4.20 a</td>
</tr>
<tr>
<td>Spray</td>
<td>0.41 a</td>
<td>2.09 a</td>
<td>0.19 a</td>
<td>2.50 a</td>
<td>3.72 a</td>
</tr>
</tbody>
</table>

*Significance n.s., not significant. Data represent mean values. In each column, means followed by the same letter are not statistically different (at P ≤ 0.05).*
ABA treatments reduce water use in peach plants, and peach and nectarine detached branches

Malus and Populus plants adapted to drought conditions show a better growth performance due to water restriction, therefore, since the peach rootstock GF-677 is a vigorous rootstock that easily adapts to unfertile and droughty soils (Reighard and Loreti, 2008), its growth could be less altered by reduced water availability.

In conclusion, ABA treatments substantially improved water retention under the considered experimental conditions while did not significantly altered biomass production. This is an interesting requirement in view of field application of the hormone under both well-watered and drought conditions.
6. CONCLUSION

In conclusion, JAs and ABA affect diverselly fruit development of peach and kiwifruits. JA-treated peach fruit confirms to be less ripe than controls, in agreement with the delayed progression of IAD, as shown by agronomical assessments and the down-regulation of crucial ripening-related ethylene genes. Moreover, both pre-harvest and post-harvest ABA treatments lead to larger, sweeter and more colored peach fruits. This is supported by the finding that ABA-treated peach trees retain more water. In kiwifruit, both hormones advance ripening by enhancing soluble sugar accumulation and flesh color development.

Also, ABA treatments substantially improved water retention under the considered experimental conditions while they did not significantly altered biomass production. This is an interesting requirement in view of field application of the hormone under both well-watered and drought conditions.

Finally, most experimental outcomes derive from studies conducted in simple and controlled environments (in vitro, growth chambers or greenhouses), and often these result in partially overlapping, but not coinciding, effects. The present information, arising from plant growth application in a field environment, where plants have to cope with multiple biotic and abiotic stresses, may open up new perspectives for the use of these substances in the control of fruit ripening.
7. REFERENCES


Bartel B. and Fink G. R. 1995. ILR1, an amidohydrolase that releases active indole-3-acetic acid from conjugates. Science 268: 1745–1748.


Kondo S., Motoyama M., Michiyama H. and Kim M. 2002. Roles of jasmonic acid in the development of sweet cherries as measured from fruit or disc samples. Plant Growth Regul. 37: 37-44.


Quiroga A. M., Berli F. J., Moreno D., Cavagnaro J. B. And Bottini R. 2009. Abscisic acid sprays significantly increase yield per plant in vineyard-grown wine grape (*Vitis vinifera* L.) cv. Cabernet Sauvignon through increased berry set with no negative effects on anthocyanin content and total polyphenol index of both juice and wine. J. Plant Growth Regul. 28: 28-35.


References


